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# CANADIAN JOURNAL OF PLANT SCIENCE

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VOLUME 40 APRIL 1960 No. 2

# INFLUENCE OF CULTURE SOLUTION TEMPERATURE ON UPTAKE OF PHOSPHORUS BY FLUE-CURED TOBACCO PLANTS[1]

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[Received for publication June 26, 1959]

## ABSTRACT

The influence of the temperature of the culture medium on the uptake of phosphorus by flue-cured tobacco plants was studied in solution culture, using radioactive phosphorus. $P^{32}$ uptake increased significantly with temperature increase from 10° to 35°C. for absorption periods of 2, 4, and 6 days. There was a further increase in $P^{32}$ uptake at 40°C. for absorption periods of 2 and 4 days but a decrease after 4 days. Increase in root growth during the absorption periods bore no relation to increase in $P^{32}$ uptake with increasing temperature.

## INTRODUCTION

It is well known that nutrient absorption and accumulation from the culture medium by roots of plants are influenced by environmental factors. Temperature has a marked effect on salt accumulation by excised barley roots (4). The absorption of potassium and calcium by barley, bean, and citrus plants is affected by root temperature (7), and soil temperature affects the uptake of phosphorus by sunflowers (2). No reference to temperature and phosphorus uptake by tobacco plants was found in the literature. Therefore, this investigation was conducted to study the effect of temperature of the culture medium on the uptake of phosphorus by tobacco plants.

## MATERIAL AND METHODS

### *Description of Materials and Equipment*

The Hicks variety of flue-cured tobacco was used in the experiments. The seedlings were grown under greenhouse conditions in silica sand in 3-inch pots and were supplied with nutrient solution. The plants were later transferred to aerated nutrient solution in glass quart jars provided with paraffined corks with holes for the support of the plants, and covered with aluminum foil to protect the roots from the light and prevent algal growth. One plant was grown per jar, and the jars were suspended in controlled-temperature tanks during the temperature tests. The plants used were of two size groups: small and medium size, ranging, respectively, from 28 to 33 cm., and 38 to 48 cm., in height from the root crown to the terminal bud.

The plants were given a complete nutrient solution previously developed for tobacco (5). The reagent grade salts $Ca(NO_3)_2 \cdot 4H_2O$,

[1]Contribution No. 19 from the Plant Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ont.
[2]Plant Nutritionist.

$KH_2PO_4$, and $MgSO_4 . 7H_2O$ supplied the major nutrient elements Ca, Mg, K, N, S, and P at concentrations of 240, 45, 195, 168, 60, and 155 p.p.m., respectively. The minor elements B, Mn, Cu, and Zn were added to the solution at the rate of 0.5 p.p.m.; Mo at the rate of 0.05 p.p.m.; and Fe, as a chleate, at the rate of 3 p.p.m. This solution was used in culturing the plants both before and during the temperature tests. However, radioactive phosphorus ($P^{32}$) was added as $NaH_2PO_4$ in tracer amounts to the nutrient solution used in the tests. This did not alter the basic composition of the solution significantly.

The tests were conducted in a room from which sunlight was excluded and the plants were illuminated with incandescent lights at an intensity of 700 foot-candles at the top of the plants for a daily photoperiod of 13 hours. The air temperature was $25 \pm 1.5°C$.

Samples of both leaf tissue and nutrient solutions were assayed for radioactivity with a thin-end-window, gas-flow counter.

*Design of Experiments*

In a series of four experiments, plants of both sizes were tested for a period of 6 days at two temperature ranges: 10°, 20°, and 30°C.; and 25°, 30°, 35°, and 40°C. In a second series of four experiments, plants of both sizes were tested at the same temperature ranges for a period of 8 hours. There were four single-plant replications at each temperature in each experiment.

*Procedure*

Prior to each experiment, the plants were preconditioned by suspending the jars in which the plants were growing in the controlled-temperature tanks. Then, over a period of 24 hours, the temperature of each tank was gradually adjusted from ambient temperature to the level at which the plants were to be grown in the experiment. The test nutrient solutions were similarly equilibrated for each temperature.

At the beginning of each 6-day experimental period, the nutrient solution was discarded from the culture jars and replaced with nutrient solution to which was added $P^{32}$ at an activity of approximately 30,000 counts per minute per ml. The volume of the labelled nutrient solution was 700 ml. per plant, and distilled water was added as required to maintain a constant volume during the 6-day absorption period. The nutrient solution supplied to all plants had an initial pH value of 4.7, which was maintained throughout the experimental period by the addition of $H_2SO_4$ as required.

At the end of the 6-day absorption period, the roots of each plant were rinsed successively in 1.0 N HCl for 15 seconds and in distilled water for 15 seconds and the rinse solutions were added to the residual nutrient solution. This was done to remove any $P^{32}$ adsorbed on the roots. Separate aliquots of both the initial and the residual nutrient solutions were evaporated in aluminum sample pans and the residue assayed for radioactivity. The difference between the total radioactivity of the initial and the residual solutions, corrected for background radiation and decay, was a measure of the total root absorption of radioactive phosphorus.

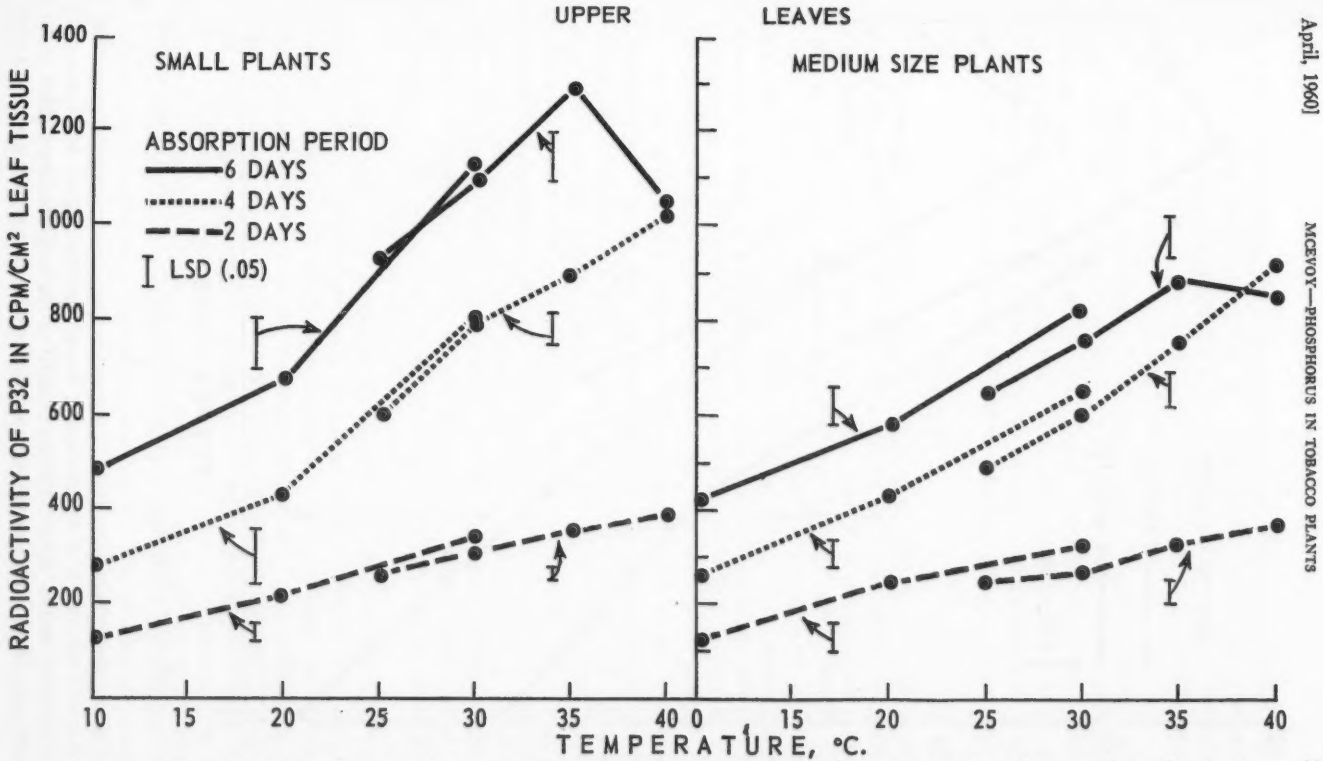


FIGURE 1. $P^{32}$ uptake by small and medium-size tobacco plants as influenced by temperature of the culture medium after 2-, 4-, and 6-day absorption periods.

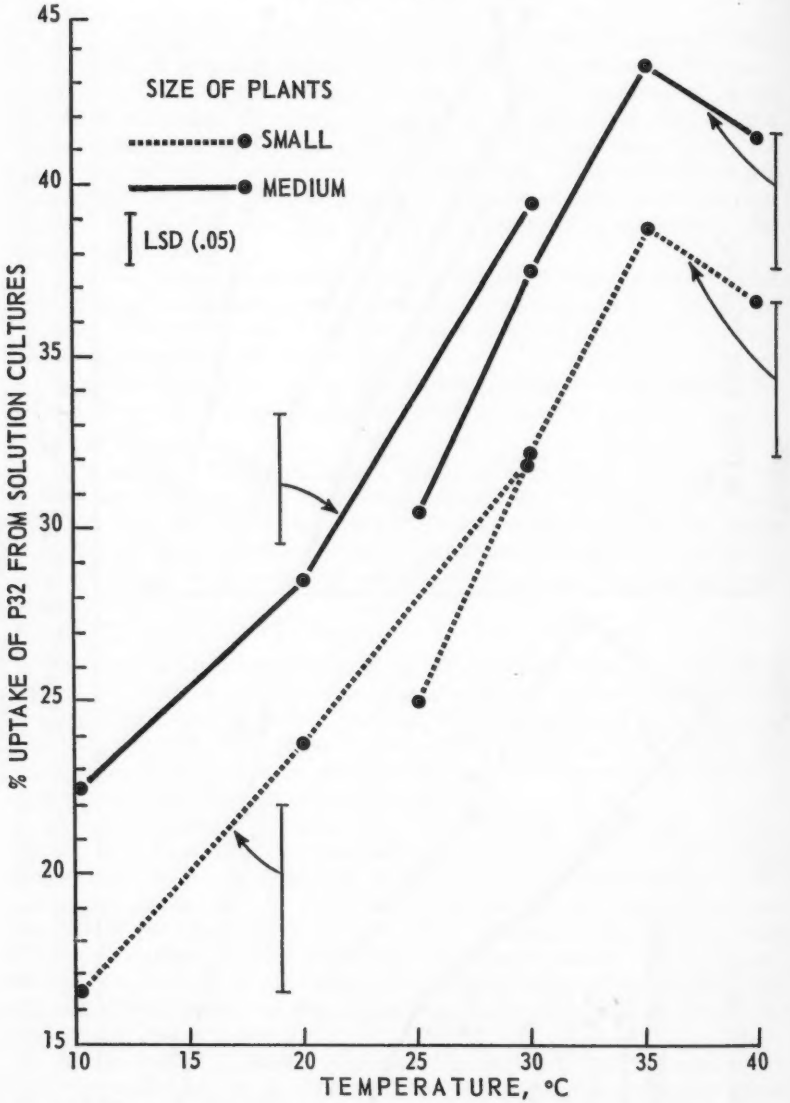


FIGURE 2. Effect of temperature on percentage absorption of $P^{32}$ from culture medium.

After absorption periods of 2, 4, and 6 days, tissue samples, consisting of discs of an area of 1 sq. cm. each, were punched out of the leaves for radioactivity assay. From an upper leaf of each plant, four discs were taken, two from each side of the midrib, one from the apex and one from

the base. The samples were attached to plastic discs with rubber cement and dried under a 150-watt lamp for 90 minutes. Radioactivity measurements were made directly on the tissue samples as a measure of the uptake of $P^{32}$. The activity of the $P^{32}$ in these samples was calculated as average counts per minute per sq. cm. of the four samples per leaf.

In sampling the leaf tissue, all but the smallest veins were avoided as the larger veins had higher $P^{32}$ activity. The samples were very uniform in thickness since they were taken from leaves of similar size from plants of the same age. Sampling was always done at the same time of day to provide uniform intervals of 2 days between successive samplings and to avoid any possible effects of diurnal variation in uptake of $P^{32}$.

## RESULTS

The results presented in Figure 1 show that, with increase in temperature of the culture medium over the range 10°, 20°, and 30°C., the increase in $P^{32}$ activity was significant at the 1 per cent level of probability in the leaves of both small and medium size plants for absorption periods of 2, 4, and 6 days. This relationship is supported by the data on percentage absorption of $P^{32}$ from the culture solutions (Figure 2) which show a significant increase in $P^{32}$ absorption with each increment in temperature. Furthermore, the $P^{32}$ uptake increased markedly with time as shown by the data on radioactivity in the leaf tissue for the three absorption periods.

In the experiment with the higher temperature range, the results (Figure 1) for 2 and 4 days for plants of both sizes show a similar pattern, namely, a positive relationship between $P^{32}$ activity in the leaf tissue and temperature of the culture medium, significant at the 1 per cent level of probability. Moreover, the effect of temperature on the percentage $P^{32}$ absorption from the culture solutions during 6 days (Figure 2) was highly significant for plants of both sizes. However, the curves for the 6-day absorption period (Figures 1 and 2) go through a maximum with the greatest $P^{32}$ uptake at 35°C. or an intermediate temperature. Thus, the efficiency of $P^{32}$ absorption by the root system decreased at 40°C. when the absorption period was extended beyond 4 days.

Consideration was given to the possibility that variation in root-absorption area concomitant with variation in root growth over the temperature ranges had a significant influence on $P^{32}$ uptake. As a test of this possible relationship, plants were supplied with $P^{32}$ at a sufficiently high rate of activity to make possible the measurement of radioactivity in the leaf tissue after an absorption period so brief that development of a significant variation in root growth among temperature treatments was improbable. To achieve this, plants of both sizes were grown as previously, except that they were removed from the radioactive culture solution after only 8 hours' exposure. Adequate uptake of $P^{32}$ was ensured by using a culture solution at an activity of approximately 130,000 counts per minute per ml. The total fresh weights of the plants were recorded at the beginning and the end of this period. The results presented in Table 1 show that the increase in $P^{32}$ uptake with increasing temperature was highly significant

TABLE 1. — ACTIVITY OF $P^{32}$ OF TOBACCO LEAF TISSUE AND GROWTH OF PLANTS AFTER AN 8-HOUR ABSORPTION PERIOD

| Temperature of culture medium | Small plants | | | | Medium size plants | | | |
|---|---|---|---|---|---|---|---|---|
| | $P^{32}$ activity per sq. cm. | | Growth per plant, fresh weight | | $P^{32}$ activity per sq. cm. | | Growth per plant, fresh weight | |
| °C. | Upper leaves | Middle leaves | gm. | % | Upper leaves | Middle leaves | gm. | % |
| | cpm. | cpm. | | | cpm. | cpm. | | |
| 10 | 258 | 192 | 2.5 | 1.9 | 224 | 174 | 2.6 | 1.8 |
| 20 | 362 | 262 | 2.6 | 3.0 | 345 | 258 | 2.9 | 1.9 |
| 30 | 511 | 400 | 3.1 | 2.4 | 476 | 375 | 3.1 | 2.2 |
| L.S.D. 5% | 74 | 47 | N.S. | N.S. | 71 | 43 | N.S. | N.S. |
| 1% | 167 | 67 | N.S. | N.S. | 102 | 62 | N.S. | N.S. |
| 25 | 402 | 305 | 3.0 | 2.3 | 372 | 302 | 3.4 | 2.2 |
| 30 | 517 | 395 | 3.4 | 2.6 | 488 | 365 | 3.5 | 2.4 |
| 35 | 664 | 612 | 3.4 | 2.6 | 632 | 596 | 3.4 | 2.3 |
| 40 | 821 | 735 | 3.3 | 2.6 | 784 | 699 | 3.4 | 2.2 |
| L.S.D. 5% | 101 | 84 | N.S. | N.S. | 129 | 77 | N.S. | N.S. |
| 1% | 142 | 117 | N.S. | N.S. | 180 | 106 | N.S. | N.S. |

in plants of both sizes. In contrast, the variation in growth of the plants associated with temperature during the 8-hour period was of a very low order, being non-significant at the 5 per cent level. Thus, growth did not have a significant effect on $P^{32}$ uptake.

## DISCUSSION

Radioactivity of leaf samples of uniform thickness as used in these studies is a valid measure of $P^{32}$ uptake (Comar, 1). In addition, Prat (6) found that, within limits, the absorption of beta rays by a great variety of leaves is a linear function of the thickness of the sample.

The effect of the temperature of the medium surrounding the roots to the content of phosphorus in the tobacco plant is shown by these studies. The markedly higher rate of phosphorus uptake at 30° and 35°C. than at lower temperatures indicates that the uptake of phosphorus by the tobacco crop will be reduced significantly by low soil temperatures.

The results of these studies are in agreement with the finding that phosphorus uptake by sunflowers grown in soil was greater at 25° to 35°C. than at 13° to 17.5°C. (2). Similar effects with other nutrient ions have been reported. The uptake of radioactive calcium by bean, barley and citrus plants increased with increase in root temperature over the range 7°, 17°, and 27°C. when grown in a single salt solution of $Ca^{45}Cl_2$ for 36 hours (7). Also, the absorption of potassium and nitrate by excised barley roots increased over the temperature range 6° to 30°C. (4).

The results indicate that the temperature effect on $P^{32}$ uptake cannot be dissociated from the duration of exposure. The exact nature of the

time factor which became effective in causing a reduction in $P^{32}$ uptake at high temperature is not known. One possible explanation is injury to the protoplasm and consequent inactivation of the enzymes in the root tissue. Thomas *et al.* (8) state that temperatures above 35°C. cause gradual inactivation of respiratory enzymes in plants. Working with tobacco plants in sand culture, Harris and van Bavel (3) found that the uptake of $P^{32}$ decreased when the concentration of oxygen in a gas mixture forced through the rooting medium dropped below 10 per cent, indicating a direct relation between rate of respiration and rate of $P^{32}$ uptake. In the present study, it seems probable that a progressive increase in the activity of the respiratory enzymes in the root tissue with temperature increase over the range 10° to 35°C. and consequent increase in the rate of respiration resulted in an increase in the rate of $P^{32}$ uptake. However, at 40°C. after the initial increase in the rate of $P^{32}$ uptake, a gradual inactivation of the respiratory enzymes resulted in a decrease in the rate of $P^{32}$ uptake.

**ACKNOWLEDGEMENT**

Thanks for advice and suggestions are extended to L. P. Dugal, Chairman, Department of Biology, University of Ottawa, Ottawa, Ont.

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# EFFECTS OF RYANIA AND RYANODINE ON THE APPARENT PHOTOSYNTHESIS OF McINTOSH APPLE LEAVES[1]

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[Received for publication January 30, 1959]

## ABSTRACT

The effects of ryania, ryanodine and ryanodine-free-ryania (powdered wood) on the apparent photosynthesis of attached apple leaves have been determined by following the $CO_2$ exchange of the leaves with an infrared gas analyser.

After one application, ryanodine decreased photosynthesis by 15 per cent but the photosynthesis had returned to normal within 7 days of treatment. Four applications of ryanodine at 10-day intervals caused no further decrease in photosynthesis than was obtained by a single application.

Ryania and powdered wood progressively reduced photosynthesis with each of four applications at 10-day intervals, until photosynthesis was reduced 40 per cent.

These results indicate that ryanodine has no marked chemical effect on apple leaves but heavy deposits of either ryania or powdered wood on the apple leaves either shade or interfere with the gas exchange of the leaves.

The rates of photosynthesis of untreated apple leaves reached a maximum of 14.95 mg. $CO_2/dm.^2 \times$ hr. when the leaves were 92 per cent of full leaf expansion but had decreased when the leaves were fully expanded and continued to decrease during senescence.

## INTRODUCTION

The botanical insecticide, ryania, is the powdered stem wood of *Ryania speciosa* Vahl, a shrub native to Trinidad and the Amazon basin. Ryania has been found effective for the control of the European corn borer, *Ostrinia nubilalis* (Hübner) (6), the sugar-cane borer, *Diatraea saccharalis* (F.), (3), the cranberry fruitworm, *Acrobasis vaccinii* Riley, (1) and the oriental fruit moth, *Grapholitha molesta* Busck, (11). When included in the spray program for apple trees, ryania gave excellent control of codling moth, *Carpocapsa pomonella*., white apple leafhopper, *Typhlocyba pomaria* Mc A., and eastern tent caterpillar, *Malacosoma americanum* (F.) in Nova Scotia (5), but in British Columbia the control of codling moth was not as good as with DDT and the apple fruits were inferior in grade and storage quality (4, 7).

This injury to the apples may be caused by reduced photosynthesis of the leaves from either a physical effect of a deposit of ryania wood particles on the leaf surfaces or a chemical effect of ryanodine, the active insecticidal ingredient of ryania, on the leaf tissues. The effects of ryania, ryanodine and ryanodine-free-ryania on the rates of apparent photosynthesis of attached apple leaves have been determined by following the $CO_2$ exchange of the leaves with an infrared gas analyser.

Two experiments are reported in this paper. In the first experiment, one application of either ryania or ryanodine was given, and the daily rates of photosynthesis were determined for 7 days after treatment. In the

[1]Contribution No. 42 from the Plant Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ont.

[2]Plant Physiologist, Plant Research Institute.

second experiment, four applications of ryania, ryanodine or ryanodine-free-ryania were given at approximately 10-day intervals. Three leaves were used for each treatment and three determinations of the rates of photosynthesis of each leaf were made between 7 and 10 days after each treatment. To avoid $CO_2$ being the limiting factor for photosynthesis it was necessary to determine the $CO_2$ exchange of single attached leaves and time did not permit more than three leaves being used for each of the four treatments. The mean rate of photosynthesis before treatment for the twelve leaves was 12.08 ± 1.395 mg. $CO_2$ uptake per $dm.^2$ of leaf area per hour giving a coefficient of variation of 11.55 per cent. This coefficient of variation was considered adequately low to show significant differences between treatments.

## MATERIALS AND METHODS

Two-year-old McIntosh apple trees on Quaker Beauty root stocks were taken from cold storage in March, transplanted into 10-inch unglazed pots containing John Innes' potting soil and placed in a growth room at 10°C., a relative humidity of 70 per cent and continuous light of 200 ft-c. After 2 weeks the leaf buds had begun to open and the trees were given 2500 ft-c of light from cool white fluorescent lamps for 18 hours at 21°C. and 6 hours of dark at 17°C. daily, at a relative humidity of 55 per cent. By 5 weeks from the time of transplanting the trees, the eighth leaf from the apex of each shoot was almost fully expanded and was used for studying the $CO_2$ exchange. By the end of the experiment in which four sprays were given, the eighth leaf had become the eighteenth leaf from the apex of the shoot.

The ryania was supplied by S. B. Penick & Company as "Ryanicide 100". A 0.6 per cent aqueous suspension of ryania, containing approximately 15 p.p.m. of ryanodine, was used for ryania treatments. For ryanodine treatments, the alkaloids, chiefly ryanodine, were extracted from the Ryanicide using the chloroform extraction method of Rogers *et al.* (9) and the concentrations in aqueous solutions were determined spectrophotometrically. In agreement with Rogers *et al.* the maximum absorption of this aqueous solution of ryanodine was at 268.5 mμ. Ryanodine-free-ryania (known in future as wood) was obtained by refluxing 10 grams of ryania in a Soxhlet apparatus for 4 days with distilled water that was changed twice each day. A 0.6 per cent aqueous suspension of this wood was sprayed on the leaves.

Leaf chambers were made from 20 x 100 mm. petri dish covers resting on Petroff culture flasks and fitted with an inlet and an outlet tube on opposite sides. Two notches in the cover accommodated the petiole and a thermocouple. The edge of the chamber was sealed with plasticine. The leaf was supported by a wire grid. A baffle in front of the inlet tube caused the air to spread out in the chamber. The temperature in the leaf chamber was maintained at 25 ± 1°C. by regulating the flow of cold water through the Petroff culture flask and the temperature was measured by a copper-constantan thermocouple pressed against the lower surface of the leaf. The relative humidity was maintained at 50 to 70 per cent by passing

the intake air through a series of four humidifiers containing concentrated solutions of ammonium chloride.

The photosynthesis was determined by measuring the $CO_2$ exchange of the attached leaves with an infrared gas analyser. This gas analyser is similar to the one used by Scarth and Shaw (10) but with a Beckman photopen recorder and an automatic air line switching device added, and is sensitive to 2 p.p.m. of $CO_2$ in atmospheric air. An attached leaf was placed in a leaf chamber through which atmospheric air was passing at a flow of 1 to 2 litres per hour per sq. cm. of leaf area. Before the rate of photosynthesis was determined, the leaf was illuminated with 2500 ft-c of fluorescent light for 30 minutes to permit the leaf to adjust to the leaf chamber conditions. At least three determinations of the rate of photosynthesis were made. A blueprint of each leaf was made on ozalid paper each day when the leaf was removed from the chamber, and the area of the blueprint was measured with a planimeter.

The sprays were applied with a non-continuous DeVilbiss No. 15 atomizer. Both the upper and lower surfaces of the leaves were sprayed until the spray ran off. All leaves of the shoot were sprayed, although only the eighth leaf from the apex of the shoot was used to study the $CO_2$ exchange. The control leaves were sprayed with distilled water. As it took 3 days to study the $CO_2$ exchange of the 12 leaves, the appropriate spray was applied each day as the leaves were removed from the leaf chambers.

To establish a relationship between the $CO_2$ exchange of the leaves reserved for later spray treatment (test leaves) and the control leaves, the

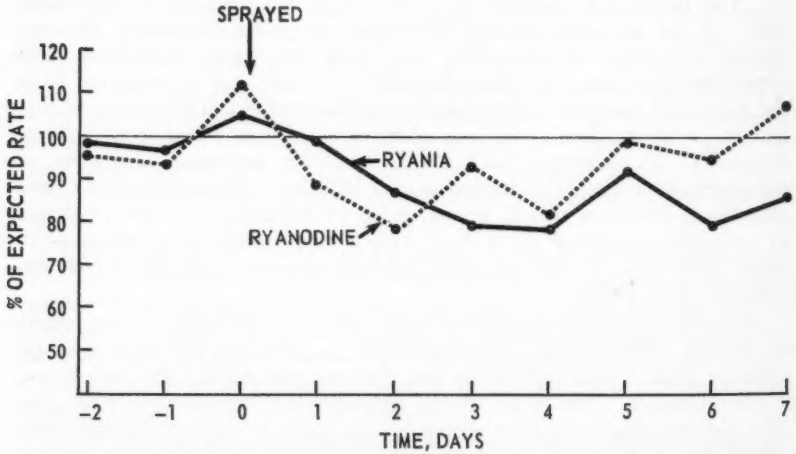


FIGURE 1. Effects of one application of ryania or ryanodine on the apparent photosynthesis of attached apple leaves. Vertical arrow indicates time of application of sprays. Rates of photosynthesis were taken daily. Each point represents the mean of one determination on each of three leaves. Mean rate of photosynthesis for the control leaves during the experiment was 6.2 ± 0.43 mg. $CO_2$ uptake per dm.$^2$ per hr.

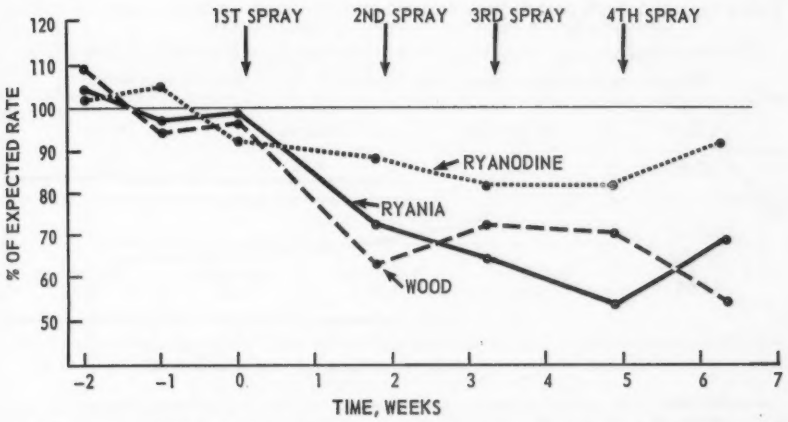


FIGURE 2. Effects of four applications at 10-day intervals of ryania, ryanodine or wood on the apparent photosynthesis of attached apple leaves. Vertical arrows indicate times of application of sprays. Rates of photosynthesis were taken between 7 and 10 days after application of the sprays. Each point represents the mean of three determinations on each of three leaves. The rates of photosynthesis of the control leaves decreased linearly during the 8 weeks of the experiment, from 13.1 to 11.4 mg. of $CO_2$ uptake per $dm.^2$ per hr.

rates of photosynthesis were determined for each of the leaves on three different days prior to spray treatment. The sprays were then applied to the leaves of the test plants, and the $CO_2$ exchange for both the test and control plants was determined for the duration of the experiment. The ratio of the rates of $CO_2$ exchange of the test and control leaves for each day is called the "expected rate". The percentage of the expected rate was calculated by using the mean of the ratios obtained before spray treatment. It was assumed that the expected rate would remain constant throughout the experiment and any variations from this expected rate after spraying would be caused by the spray on the leaves. When the photosynthesis of two sets of two non-sprayed leaves was followed for 8 weeks, the standard deviation of the expected rate was ±9.9 per cent of the mean. This standard deviation was sufficiently low for the purpose of the experiment.

## RESULTS AND DISCUSSION

### *One Application of Ryania or Ryanodine*

Figure 1 gives the time course for the apparent photosynthesis of apple leaves sprayed with a 0.6 per cent aqueous suspension of ryania or an aqueous solution containing 90 p.p.m. of ryanodine. The photosynthesis of leaves sprayed with either ryania or ryanodine decreased to 80 or 85 per cent of the expected rate by the second day after treatment. The photosynthesis of the ryania-treated leaves remained around this level while that of the ryanodine-treated leaves gradually returned to normal by the seventh day.

TABLE I. — DUNCAN'S TEST OF SIGNIFICANCE OF DIFFERENCE BETWEEN TYPES OF SPRAYS AND DIFFERENCE BETWEEN NUMBER OF TIMES SPRAYED.

(Any two rates joined by the same line do not differ significantly at the 5 per cent level.)

Mean rates of photosynthesis, mg. $CO_2$/dm.$^2$ x hr. in order of magnitude.

| Control | Ryanodine | Ryania | Wood |
|---|---|---|---|
| 12.02 | 10.24 | 8.14 | 7.83 |

| Before spray | After | | | |
|---|---|---|---|---|
| 12.08 | 1st spray 10.15 | 3rd spray 8.71 | 4th spray 8.62 | 2nd spray 8.23 |

Analysis of data for ryania and wood

| Before spray | After | | | |
|---|---|---|---|---|
| 11.68 | 1st spray 8.16 | 2nd spray 6.82 | 3rd spray 6.78 | 4th spray 6.49 |

An analysis of variance on the actual rates of apparent photosynthesis in mg. $CO_2$ uptake / dm.$^2$ x hr. for days 2, 3, and 4 showed that there were no significant differences between the test leaves and the controls. These results indicate that one application of either ryania or ryanodine causes no serious injury to apple leaves. Also, in a spray program where four applications of ryania are given at 10-day intervals there would be time for the photosynthesis to return to normal before the next spray was applied.

*Four Applications of Ryania, Ryanodine or Wood*

Figure 2 gives the time course for the apparent photosynthesis of apple leaves sprayed four times with a 0.6 per cent aqueous suspension of ryania or of wood, or an aqueous solution of 30 p.p.m. of ryanodine. An analysis of variance was carried out on the rates of photosynthesis, separating the effects of type of spray and the number of times sprayed. The standard error for types of spray averaged over all the number of times sprayed was $\pm 0.475$ and the standard error for the number of times sprayed averaged over all types of spray was $\pm 0.442$. The significance of the various differences between types of sprays and between number of times sprayed as determined at the 5 per cent level in the Duncan's test (2) is shown in Table 1.

Even though the concentration of ryanodine used in this experiment was twice that found in ryania sprays used in apple orchards the photosynthesis of the ryanodine-treated leaves decreased to only 82 per cent of the expected rate. The photosynthesis of the leaves sprayed with wood dropped to around 60 per cent of the expected rate. With each additional spray of ryania and wood the photosynthesis of the leaves showed a further decrease (Table 1), indicating a cumulative effect. These results suggest that the ryanodine has no marked chemical effect on apple leaves but the

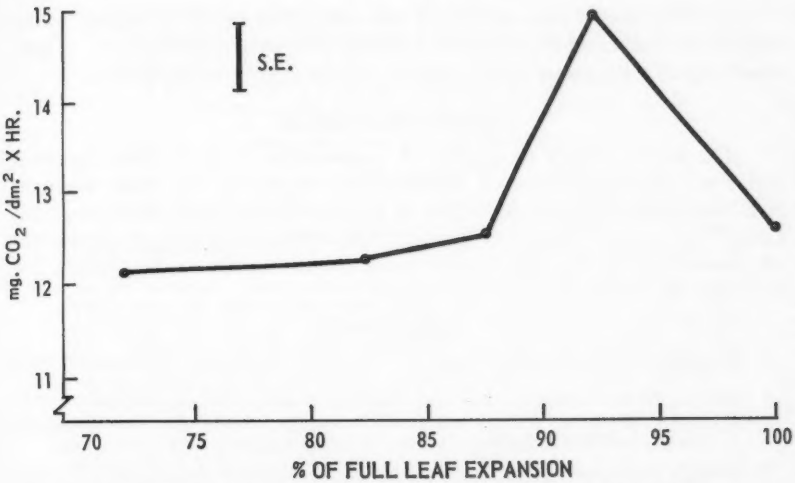


FIGURE 3. Effects of physiological age (per cent of full leaf expansion) on the rates of apparent photosynthesis of attached apple leaves. Each point is the mean of five leaves.

deposit of particles from ryania building up on the leaf surfaces was reducing photosynthesis either by shading or by partially plugging the stomata and interfering with the gas exchange of the leaves. This accumulation of particles on the leaf surfaces would be expected to occur in the Okanagan Valley, British Columbia, where there are infrequent rain showers, and it may account for the injury that has been reported from there (4). The frequent rains in Nova Scotia would tend to prevent this accumulation of the particles on the leaf surfaces.

*Effect of Physiological Age of Apple Leaves on the Rate of Apparent Photosynthesis*

Observations on the effect of age on the rate of photosynthesis of apple leaves were incidental in this work but, because of the few references in the literature on this topic (8), they are worth reporting. In the experiment where four applications of ryania were given at 10-day intervals, the photosynthesis studies were started before some of the leaves in the eighth position from the apex of the shoot had fully expanded. The percentage of full leaf expansion was used as a measure of physiological age. The effects of physiological age on the rates of photosynthesis are given in Figure 3.

As the percentage of full leaf expansion increased from 72 to 92 per cent the rates of photosynthesis increased, but when the leaves were fully expanded the rates had decreased. At 92 per cent of full leaf expansion the rates of photosynthesis were significantly higher at the 5 per cent level in the Duncan's test than the other rates, which were not significantly different. During senescence, from the time of full leaf expansion until

7 weeks later, the photosynthesis of the control leaves in the second ryania experiment decreased from 13.1 to 11.4 mg. $CO_2$ uptake/dm.$^2$ × hr. These results agree with those of Richardson (8) for *Acer saccharinum* L.

**ACKNOWLEDGEMENTS**

The author wishes to express his appreciation to D. S. Blair, Genetics and Plant Breeding Research Institute, for supplying the apple trees; to S. B. Penick & Company for a gift of the powdered ryania stem; to D. H. Laughland, Animal Research Institute, for determining the concentrations of ryanodine on the spectrophotometer, and to M. J. P. Leyritz for technical assistance.

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# DETERMINATION OF BOTANICAL COMPOSITION OF TWO-COMPONENT FORAGE MIXTURES[1]

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## ABSTRACT

A comparative study was made in 1958 of the visual estimation and hand separation methods of determining botanical composition of two-component forage mixtures. The results indicated that there were positive significant correlations between the per cent legume values obtained by the two methods. The visual estimation method was less variable than the hand separation method and the precision per unit cost was greater. The differences between per cent legume values obtained by the two methods were influenced by the stage of maturity (medium or late hay) of the components and the cut (hay or aftermath). In this study, the difference was significant only in the medium aftermath cut.

Individually, three observers showed some inconsistencies between estimates on the medium and late maturity groups and between the hay and aftermath cut. However, by averaging the three estimates to obtain a mean sample, these inconsistencies were minimized.

Both methods were more precise in the aftermath pasture cut than in the hay. An additional observer increased precision of the visual estimate more than an additional replicate or sample. The greater precision resulting from additional replicates, samples, or observers increased at a decreasing rate. The number of replicates, samples, and observers required for specific degrees of precision and a specific cost were calculated.

The experiment showed that the visual estimation method can be superior to the hand separation method as a means of determining botanical composition.

## INTRODUCTION

Forage investigators frequently must determine botanical composition of mixtures. The hand separation method is usually accepted as the most precise method of determining botanical composition although most investigators (1, 2, 6, 9) agree that the method is time-consuming and laborious. The importance of a rapid, inexpensive, alternate method which could, in part, replace hand separations, cannot be over-emphasized. Visual estimation of the standing crop is the most rapid and inexpensive method. However, certain aspects of the reliability of this method have been investigated only sparsely (3, 4, 6, 8).

This study was initiated to investigate the visual estimation method more intensively in an attempt to have a better evaluation of the method when compared to the hand separation method.

## MATERIALS AND METHODS

The study was conducted in 1958 on a timothy, *Phleum pratense* L., strain test which was seeded in 1956. The test contained six timothy varieties, Common, Climax, Drummond, Essex, S-48, and S-51, representing a range of maturities. These were overseeded with Vernal alfalfa, *Medicago sativa* L.

[1]Condensation of a thesis submitted to the Graduate School, University of Toronto, by J. W. Tanner, in partial fulfilment of the requirements for the Master of Science in Agriculture.

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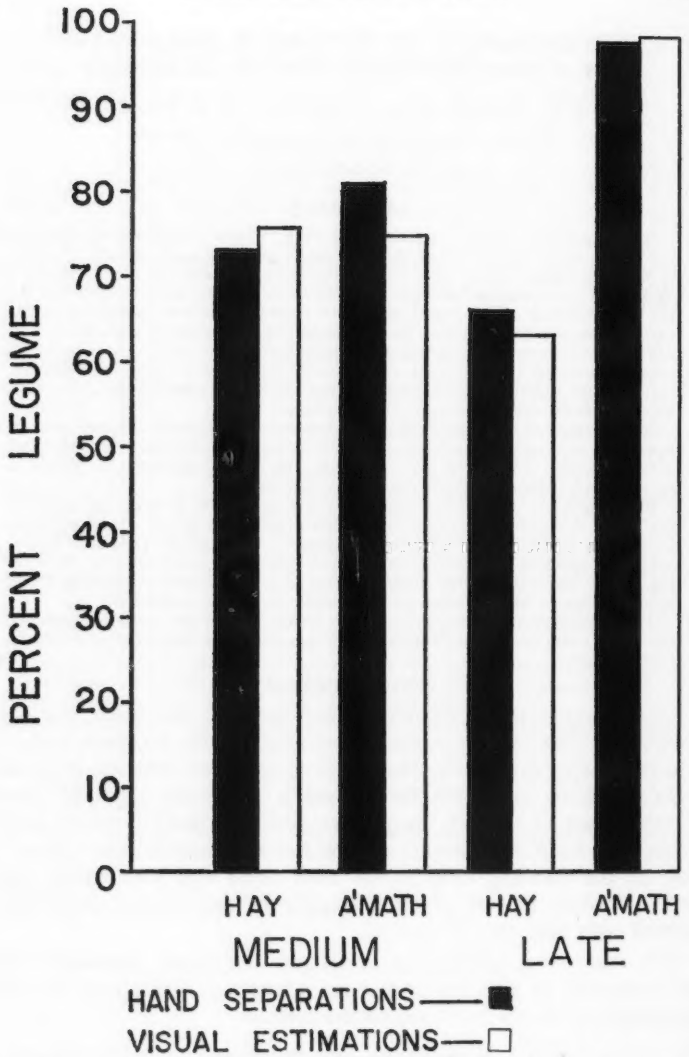


FIGURE 1. Per cent legume obtained by hand separation and visual estimation on medium and late hay and aftermath illustrating method x maturity x cut interaction (retransformed means used).

The experiment was divided into two maturity groups with respect to harvesting dates, medium and late. In the medium maturity group all six varieties were cut when the earliest variety, Common, was at the full bloom stage. In the late maturity group all six varieties were cut when S-48 and S-51 were at the full bloom stage. A hay cut and an aftermath pasture

cut were taken from each plot within a group. There were two methods of determining botanical composition used and two samples taken in each method, the unit of observation in all cases being per cent legume.

The experiment was set up in a split-plot design with maturity groups constituting the first split. The second split consisted of varieties; the third, cuts; and the final split, methods. The plots were 22 feet by 5 feet and were seeded broadcast at a rate of 8 pounds of timothy and 6 pounds of alfalfa per acre.

TABLE 1. — ANALYSIS OF VARIANCE OF PER CENT LEGUME[1] FOR TOTAL (MEDIUM HAY + LATE HAY + MEDIUM AFTERMATH + LATE AFTERMATH)

| Variation due to: | Degrees of freedom | Mean square |
|---|---|---|
| Replicates | 3 | 635.6167 |
| Maturity | 1 | 131.5783 |
| Error (a) | 3 | 86.0775 |
| Varieties | 5 | 314.5954** |
| Varieties x maturities | 5 | 23.6972 |
| Error (b) | 30 | 28.01 |
| Cuts | 1 | 51,683.5689** |
| Maturity x cuts | 1 | 2,498.7962** |
| Variety x cuts | 5 | 24.2442 |
| Variety x cuts x maturity | 5 | 7.1905 |
| Error (c) | 36 | 17.0638 |
| Methods | 1 | 264.4381** |
| Methods x maturity | 1 | 140.7957** |
| Methods x variety | 5 | 21.8425 |
| Methods x cuts | 1 | 239.4015** |
| Methods x variety x cuts | 5 | 4.1505 |
| Methods x maturity x cuts | 1 | 824.6780** |
| Methods x variety x maturity | 5 | 12.6716 |
| Variety x methods x maturity x cuts | 5 | 2.6736 |
| Error (d) | 72 | 15.4310 |
| Samples | 192 | 3.5141 |
| Total | 383 | |

[1]Transformed data using arcsin transformation
**Significant at the 1 per cent level

TABLE 2. — COEFFICIENTS OF VARIATION AND MEAN SQUARES FOR ANALYSES OF VARIANCE CALCULATED FROM METHODS WITHIN CUTS

| Treatment | Coefficient of variation | | Mean square | |
|---|---|---|---|---|
| | Sample 1 | Sample 2 | Sample | Error |
| Hay, visual estimation | 5.27 | 4.12 | 1.56 | 13.14 |
| Hay, hand separation | 8.63 | 9.37 | 8.91 | 40.88 |
| Aftermath, visual estimation | 2.16 | 1.90 | 1.43 | 3.45 |
| Aftermath, hand separation | 2.33 | 2.57 | 2.61 | 6.41 |

Two samples of fresh material for hand separation, each of approximately 1,000 grams, were obtained from each plot by collecting a random sample from a 3′ x 18′ yield strip in the manner described by Peterson and Chamblee (5). The samples were held at 28°F. until separated into botanical components and then the per cent legume was calculated on a dry matter basis.

In the visual estimation method, each of three observers made one independent estimate of per cent legume in each plot. The three estimates were combined to give a mean estimate which, in this experiment, constitutes a sample. Two samples were obtained from each plot, one on the day preceding harvesting and the other on the day of harvest.

Statistical analyses were carried out to determine whether the methods gave significantly different results. All percentages were transformed using the arcsin transformation as described by Snedecor (7). Correlation coefficients between methods were calculated for the medium hay, late hay, medium aftermath, late aftermath cuts and for the total of all four treatments. Analyses of variance were computed for each sample within each method and within each cut. The coefficient variability (C.V.) from each of the above eight analyses was transformed logarithmically and analysed for differences by means of the analysis of variance technique.

An analysis of variance was computed for each method within each cut. Error variances from these analyses were used in calculating variances of the treatment mean ($s_{\bar{y}}^2$) in the manner described by Snedecor (7). The variances of the treatment mean were used as a measure of the precision of each method, and were calculated for varying numbers of replicates and samples. Appropriate cost factors were calculated for the corresponding variables. Plot cost included land values and cost of fertilizer, tillage, seed and labour involved in establishing a plot. The cost of a hand separation sample included the cost of obtaining, freezing, separating, drying, and weighing the sample. The cost of a visual estimation was equal to the value of time required by three observers to estimate one plot.

An analysis of variance was computed on visual estimations alone in order to investigate differences between observers. Variances of the treatment means were calculated under hay and aftermath pasture for the visual estimate method for varying numbers of replicates, samples and observers. The corresponding cost factors were calculated.

## EXPERIMENTAL RESULTS AND DISCUSSION

The analysis of variance in Table 1 indicates significant interactions involving methods. The methods x maturities x cuts interaction is illustrated in Figure 1. In order to determine the main source of this interaction, t-values were calculated for the four treatments, medium hay, late hay, medium aftermath, and late aftermath. These showed that the two methods provided similar estimates of legume percentage in all cases except on the medium maturity aftermath pasture cut. In this case the hand separation values were significantly higher than the visual estimation values. The non-significant methods x varieties interaction indicates that the two methods are equally effective on all varieties.

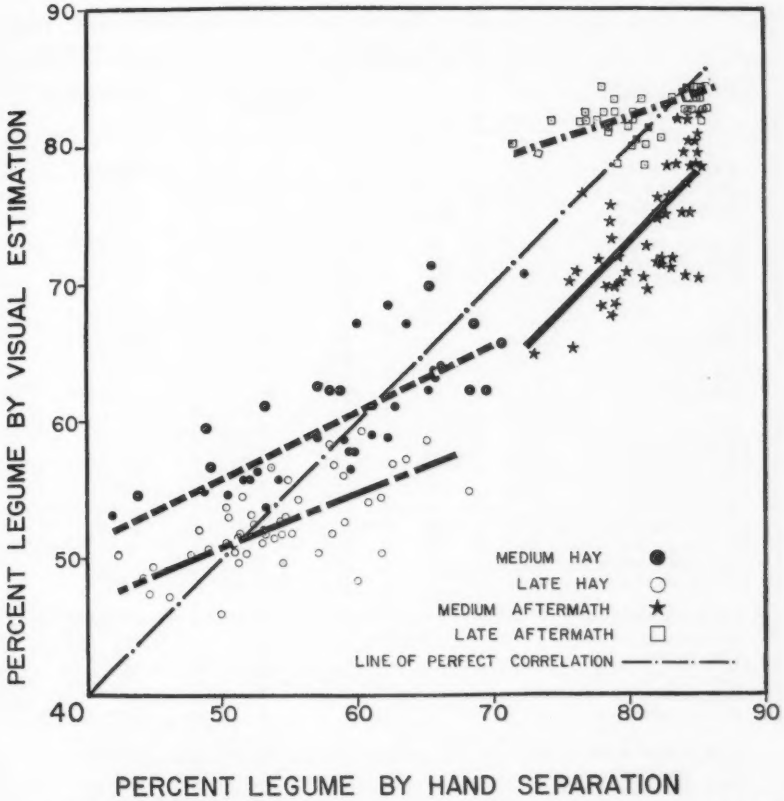


FIGURE 2. Scatter diagram and regression line for hand separations and visual estimations and the line of perfect correlation ($r = 1$).

The legume percentage estimates from the two methods were closely associated giving highly significant and positive correlation coefficients in each treatment. These ranged from $r = +.529$ to $r = +.737$. The average relationship between the two methods ($r = +.923$) calculated using all values in the four treatments was not a good estimate of the association because of the bias introduced by the wide difference in per cent legume between the hay and aftermath treatments. A better estimate of the general correlation between the two methods was obtained by means of the z-transformation (7) and this value, +.664, indicates good general association.

Correlation coefficients alone do not give an adequate comparison of the methods. When interactions, which in this study provided much of the important information, were present, they were not shown by the use of correlations. For example, the medium aftermath treatment showed a high correlation between methods but a significant difference between

TABLE 3. — ANALYSIS OF VARIANCE OF PER CENT LEGUME[1] OF TOTAL OBTAINED BY THREE VISUAL ESTIMATIONS

| Variation due to: | Degrees of freedom | Mean square |
|---|---|---|
| Replicates (reps.) | 3 | 992.21 |
| Maturity (mat.) | 1 | 10.05 |
| Error (a) | 3 | 178.83 |
| Variety (var.) | 5 | 274.95** |
| Mat. x var. | 5 | 51.69 |
| Error (b) | 30 | 32.63 |
| Cut | 1 | 68,380.30** |
| Mat. x cut | 1 | 8,966.27** |
| Var. x cut | 5 | 36.57 |
| Mat. x var. x cut | 5 | 13.33 |
| Error (c) | 36 | 24.28 |
| Samples | 1 | 1.87 |
| Samples x mat. | 1 | .11 |
| Samples x var. | 5 | 6.51 |
| Samples x cut | 1 | 18.77 |
| Samples x mat. x var. | 5 | 1.48 |
| Samples x mat. x cut | 1 | .02 |
| Samples x var. x cut | 5 | 5.11 |
| Samples x var. x mat. x cut | 5 | 1.88 |
| Error (d) | 72 | 40.79 |
| Observers (obs.) | 2 | 377.15** |
| Obs. x mat. | 2 | 642.62** |
| Obs. x var. | 10 | 14.99 |
| Obs. x mat. x var. | 10 | 13.78 |
| Obs. x cuts | 2 | 125.21** |
| Obs. x mat. x cut | 2 | 9.96 |
| Obs. x var. x cut | 10 | 15.17 |
| Obs. x mat. x var. x cut | 10 | 10.44 |
| Obs. x samples | 2 | 1.51 |
| Obs. x samples x mat. | 2 | 3.81 |
| Obs. x samples x var. | 10 | 4.17 |
| Obs. x samples x cut | 2 | .55 |
| Obs. x samples x mat. x var. | 10 | 3.33 |
| Obs. x samples x mat. x cut | 2 | 1.27 |
| Obs. x samples x var. x cut | 10 | 5.87 |
| Obs. x samples x var. x mat. x cut | 10 | 3.37 |
| Error (e) | 288 | 9.34 |

[1]Data transformed using arcsin transformation
**Significant at the 1 per cent level

methods whereas all other treatments showed significant correlation values but no difference between methods. This relationship is shown in Figure 2. Most of the points for the medium aftermath cut were below the line of perfect correlation thus indicating a bias in the visual estimations of this treatment.

Table 2 contains the coefficients of variation obtained from the analyses of variance of the samples within methods within cuts. An analyses of variance on these coefficients showed that there was a method x cuts interaction which indicated that while there was a significant difference between the coefficients of variation for the visual estimations and the hand separa-

TABLE 4. — COST FACTORS[1] AND VARIANCES OF THE TREATMENT MEANS ($s_{\bar{y}}^2$)* FOR TWO METHODS ON HAY AND AFTERMATH PASTURE FOR VARYING NUMBERS OF REPLICATES AND SAMPLES.

| Number of replicates | Number of samples[2] | Visual estimate | | | Hand separation | | |
|---|---|---|---|---|---|---|---|
| | | Cost in dollars | $s_{\bar{y}}^2$ | | Cost in dollars | $s_{\bar{y}}^2$ | |
| | | | Hay | Aftermath | | Hay | Aftermath |
| 3 | 1 | 5.10 | 2.45 | .82 | 7.80 | 8.30 | 1.43 |
| | 2 | 5.70 | 2.19 | .58 | 11.10 | 6.82 | 1.07 |
| | 3 | 6.30 | 2.10 | .49 | 14.40 | 6.32 | .95 |
| | 4 | 6.90 | 2.06 | .46 | 17.70 | 6.07 | .89 |
| 4 | 1 | 6.80 | 1.84 | .61 | 10.40 | 6.23 | 1.07 |
| | 2 | 7.60 | 1.65 | .43 | 14.80 | 5.11 | .80 |
| | 3 | 8.40 | 1.58 | .37 | 19.20 | 4.74 | .71 |
| | 4 | 9.20 | 1.55 | .34 | 23.60 | 4.56 | .67 |
| 5 | 1 | 8.50 | 1.47 | .49 | 13.00 | 5.18 | .86 |
| | 2 | 9.50 | 1.32 | .34 | 18.50 | 4.29 | .65 |
| | 3 | 10.50 | 1.26 | .30 | 24.00 | 3.99 | .57 |
| | 4 | 11.50 | 1.24 | .27 | 29.50 | 3.85 | .54 |
| 6 | 1 | 10.20 | 1.23 | .41 | 15.60 | 4.16 | .72 |
| | 2 | 11.40 | 1.10 | .29 | 22.20 | 3.41 | .54 |
| | 3 | 12.60 | 1.06 | .25 | 22.80 | 3.17 | .48 |
| | 4 | 13.80 | 1.04 | .23 | 35.40 | 3.04 | .45 |

* $s_{\bar{y}}^2 = \frac{\sigma e^2}{n_1} + \frac{\sigma s^2}{n_1 n_2}$

[1] Cost = $n_1c_1 + n_1n_2c_2$

where: $\sigma e^2$ = variance for error
$\sigma s^2$ = variance for sample
$n_1$ = number of replicates
$n_2$ = number of samples
$c_1$ = cost of plot = \$1.50
$c_2$ = cost of sample = Visual \$ .21
Hand \$1.10

[2] In the visual estimation method a sample consisted of the mean of three estimates.

tions, this difference was not the same in the hay cut as in the aftermath pasture cut. There was less variation in the visual estimation method than in the hand separation method and less variation in the aftermath than in the hay. These differences can be partly explained. In the hand separation method, there is a good possibility that when collecting a random sample, the sampler could pick up a big alfalfa plant. This would give a relatively greater amount of legume when hand separated than what was actually in the plot. In the visual estimation method, the whole plot is observed, not a sample, hence sampling error could be smaller. In the hay cut, the plants are large and there would be a greater chance of this occurring than in the aftermath when plants are smaller. Also, in the hay cut, the range of per cent legume was wider than in the aftermath pasture cut.

The analysis of variance of the data obtained by the three observers is shown in Table 3. There was no significant difference between the two

TABLE 5. — COST FACTORS[1] AND VARIANCES OF THE TREATMENT MEANS* FOR VISUAL ESTIMATIONS FOR VARYING NUMBERS OF REPLICATES, SAMPLES, AND OBSERVERS

| Number of samples | Number of observers per sample | Number of replicates | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | 3 | | 4 | | 5 | | 6 | |
| | | $s_{\bar{y}}^2$ | Cost in dollars | $s_{\bar{y}}^2$ | Cost in dollars | $s_{\bar{y}}^2$ | Cost in dollars | $s_{\bar{y}}^2$ | Cost in dollars |
| 1 | 1 | 4.88 | 4.71 | 3.66 | 6.28 | 2.93 | 7.85 | 2.45 | 9.42 |
| | 2 | 2.40 | 4.92 | 1.79 | 6.56 | 1.44 | 8.20 | 1.20 | 9.84 |
| | 3 | 1.57 | 5.13 | 1.17 | 6.84 | .94 | 8.55 | .79 | 10.26 |
| 2 | 1 | 3.01 | 4.92 | 2.25 | 6.56 | 1.80 | 8.20 | 1.50 | 9.84 |
| | 2 | 1.76 | 5.34 | 1.32 | 7.12 | 1.06 | 8.90 | .88 | 10.68 |
| | 3 | 1.35 | 5.76 | 1.01 | 7.68 | .81 | 9.60 | .67 | 11.52 |
| 3 | 1 | 2.38 | 5.13 | 1.78 | 6.84 | 1.42 | 8.55 | 1.19 | 10.26 |
| | 2 | 1.55 | 5.76 | 1.16 | 7.68 | .93 | 9.60 | .77 | 11.52 |
| | 3 | 1.27 | 6.39 | .95 | 8.52 | .76 | 10.65 | .64 | 12.78 |
| 4 | 1 | 2.06 | 5.34 | 1.54 | 7.12 | 1.24 | 8.90 | 1.03 | 10.68 |
| | 2 | 1.44 | 6.18 | 1.08 | 8.24 | .86 | 10.30 | .72 | 12.36 |
| | 3 | 1.23 | 7.02 | .92 | 9.36 | .74 | 11.70 | .62 | 14.04 |

*$s_{\bar{y}}^2 = \frac{\sigma e^2}{n_1} + \frac{\sigma s^2}{n_1 n_2} + \frac{\sigma o^2}{n_1 n_2 n_3}$

$\sigma e^2$ = variance for error (e) = 3.37
$\sigma s^2$ = variance for sampling = −3.62
$\sigma o^2$ = variance for observing = 14.91

[1] Cost = $c_1 \times n_1 + c_2 n_1 n_2 n_3$
$c_1$ = cost of plot \$1.50
$c_2$ = cost of one visual estimate \$.07

$n_1$ = number of replicates
$n_2$ = number of samples
$n_3$ = number of estimates

samples. There was a significant difference between the estimates of the three observers. However, the interactions, observers x maturities and observers x cuts, were significant indicating that the difference between observers varied depending on cut and maturity. The actual difference in the estimates between observers was not large and did not appear to be serious. When the three estimates were averaged, the resulting mean compared favourably with the hand separation value in most cases. Observer A, who was consistent in all cases, had over 30 years' experience in forage work although in that period had done very little visual estimating. Observers B and C, who had relatively little experience in forage experiments, were more variable in their estimates.

It is not unreasonable to assume that three other observers would provide slightly different results than those obtained in this experiment. However, it does appear that reliable visual estimates can be made by observers with little or no previous experience in visual estimation of legume composition. The presence of interactions involving observers indicates that care is necessary in training observers in order to avoid such interactions.

The mean squares for error and for samples listed in Table 2 were used to calculate variances of the treatment means shown in Table 4. These variances of the treatment means are estimates based on the components of variance obtained in this experiment. The precision in every comparison was greater in the visual estimation method than in the hand separation method. Both methods showed more precision in the aftermath than in the hay cut. Appropriate cost factors were also included in this table. The high cost of hand separations as compared to visual estimations is obvious in all cases.

From Table 3, the mean square for error (c), a pooled sampling error, and a pooled observing error were used to calculate the variances of the treatment mean found in Table 5. This table shows that with a given number of replicates, more precision is gained by increasing the number of observers than by increasing the number of samples. This is partially a result of the small sampling error in the experiment. Also, the use of an extra observer decreases the variance of the treatment mean more rapidly than the addition of an extra replicate and the cost of adding another observer is low compared to the cost of an extra replicate. The table gives an indication of the precision that could be expected and the cost that would be encountered for a set number of replicates, samples, and observers. Also, if the number of replicates required for precision of yield data is set, Table 5 gives an indication of the number of samples and observers that would be required for a specific degree of precision on botanical analysis.

It is emphasized that the cost factors were calculated assuming botanical composition to be the important factor under study. If, as is frequently the case, yield is the important factor, the cost factors presented here would be higher than the actual cost, because part of the plot cost would be charged to the yield experiment.

The $s_{\bar{y}}^2$ measured the precision of each method but did not indicate which method gave results most nearly approximating the actual botanical analysis of the plots. In many experiments, the per cent of a component in one plot relative to the per cent of another component in another plot is as meaningful as the actual per cent of each. If the relative amounts of the estimated are proportional to the relative amounts of the actual, the same conclusions will be drawn. The same is true for hand separations. However, the difference between visual estimates and the actual percentage probably becomes smaller as the observers become experienced. On the other hand, in the hand separation method, it is very costly to increase the size or number of samples in an attempt to reduce the deviation from the actual per cent.

Visual estimations consistently gave lower variances of the treatment mean ($s_{\bar{y}}^2$) values thus indicating that the method was more precise than the hand separation method. The greater precision coupled with the lower costs show that the precision per unit cost was much greater using visual estimations than hand separations.

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# THE EFFECTS OF VARIOUS INCREMENTS OF N, P AND K ON THE YIELD AND BOTANICAL COMPOSITION OF PERMANENT PASTURES[1]

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## ABSTRACT

Applications of N, P and K to an area grazed and unfertilized for over 30 years resulted in some immediate and marked improvements in yields and botanical composition. Phosphorus, at rates up to 80 pounds per acre, brought about the most marked improvement. This element was responsible for highly significant increases in yield, increased wild white clover and useful grasses and decreased weeds, moss and bare ground markedly. Potassium, up to 60 pounds per acre, also gave highly significant increases in yield. Potassium had relatively little effect on the prevalence of species at first, but by the fourth year had maintained white clover better than phosphorus and had also decreased weeds and moss. Nitrogen, at 15 pounds per acre, increased yields each year but only during the early part of the season. Thirty pounds per acre was superior to the 15-pound rate one year only and this was also during the early part of the season. It actually tended to decrease yields during July and August. The effect of nitrogen on botanical composition was largely to decrease the percentage of wild white clover with a corresponding increase in grasses.

## INTRODUCTION

Approximately one-sixth of the total occupied agricultural land in New Brunswick is devoted to pasture. Notwithstanding its importance a large proportion of this pasture has low productivity. Data published by the Central Experimental Farm Pasture Committee (7) showed that 3.90 acres of pasture were required to carry one animal unit throughout the grazing season in New Brunswick, compared to one acre or less on productive pastures.

Early work on the effect of surface applications of commercial fertilizer to pastures clearly indicated that outstanding improvement in yields could be obtained by that practice in New Brunswick. For instance, Bailey (1) showed that the carrying capacity of permanent pastures at Fredericton, N.B., was doubled by fertilizing with 350 pounds of 16 per cent superphosphate and 100 pounds muriate of potash every two years, plus 150 pounds of nitrate of soda each year.

The effects of surface applications of commercial fertilizers to permanent pastures have been extensively studied in other parts of Eastern Canada and the Eastern United States. Despite wide variations in the results obtained under different environmental conditions, the following trends are evident in the majority of the published reports:

(a) Phosphorus was the element most needed for pasture improvement both from the standpoint of increasing yields and improving vegetation (4, 6, 11, 12, 15, 16, 17, 18, 19, 21, 22).

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[1]Contribution No. 3 from the Research Station, Canada Department of Agriculture, Fredericton, N.B., based on thesis submitted to the School of Graduate Studies, McGill University, in partial fulfilment of requirements for the degree of M. Sc.

TABLE I — 1950 SUMMARY OF YIELD INCREASES CONTRIBUTED BY VARIOUS RATES OF N, P AND K
(Pounds of Dry Matter per Acre)

| | Date cut | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | June 7 | June 20 | July 6 | July 20 | Aug. 7 | Aug. 24 | Sept. 20 | Total for season |
| $N_1 - N_0$ | 77.1** | 36.4** | 16.3 | − 1.3 | − 4.5 | 8.7 | 14.8 | 147.8* |
| $N_2 - N_1$ | 97.8** | 22.1** | 18.3 | − 4.3 | 5.4 | 8.8 | 7.4 | 155.9* |
| $P_1 - P_0$ | 95.0** | 35.2** | 73.8** | 54.3** | 66.5** | 34.6** | 57.9** | 417.3** |
| $P_2 - P_1$ | 11.3 | 22.5** | 51.4** | 52.5** | 52.2** | 30.2** | 50.5* | 270.6** |
| $P_3 - P_2$ | 24.8 | 17.1* | 28.2 | −11.2 | 2.3 | 5.9 | 13.0 | 80.1 |
| $K_1 - K_0$ | − 8.8 | 9.4 | 36.6* | 33.2** | 43.4** | 30.0** | 26.2 | 169.9** |
| $K_2 - K_1$ | 16.6 | 13.0 | 23.0 | 26.0* | 26.2* | 12.6 | 13.3 | 130.8* |

*Significant at the 5 per cent level
**Significant at the 1 per cent level

(b) Potassium brought about marked increases in yields and improvement in vegetation especially where this element was deficient in that soil (10, 15, 18, 21). In other cases potassium had little effect on yields but increased the amount of clover (6, 11, 17).

(c) Nitrogen nearly always increased yields but in most cases brought about a decrease in the clover content of the sward (2, 4, 5, 8, 10, 11, 15, 18, 19, 20, 21). Studies conducted on the distribution of increased growth attributable to nitrogen showed that this element increased growth during the May-June period and had little effect on yields for the remainder of the season (3, 4, 8, 9, 18, 22).

The purpose of this study was to determine more fully the effect of each of the three major elements on the yield of permanent pastures, the influence of each element on the seasonal distribution of growth and their effect on the botanical composition of the sward.

## MATERIALS AND METHODS

The study herewith reported was begun in 1950 on an area of permanent pasture at the Canada Department of Agriculture Research Station, Fredericton, N.B. The area chosen was seeded down in 1918 and was grazed each year from 1923 until the beginning of the experiment. No commercial fertilizer or manure had been applied to this area since 1918. As a result the sward was rather poor and contained a large proportion of weeds of which the most common were *Leontodon autumnalis* L. and *Hieracium aurantiacum* L. A moderate number of plants of *Trifolium repens* L. were present but the plants always remained small and contributed little to the yield. *Agrostis tenuis Sibth* was the most common grass noted.

The soil is classed as Riverbank sandy loam, stony phase and is gently sloping. A description of this soil series as given by Stobbe (23) follows: "The Riverbank series occupies 4.0 per cent of the surveyed area. It is found mainly on well drained slopes in the river valleys.

"Stones and boulders are not so numerous in this series as on upland soils and they are found more frequently in the subsoil than in upper soil horizons.

"The cultivated Riverbank soils consist of light brown friable sandy loams. The reaction of unlimed soils varies from pH 5.0 to 5.5. The surface is underlain by sandy loam to sand, the colour of which changes from a reddish brown to a greyish brown and grey with greater depth. At approximately 3 feet it is underlain by compacted boulder clay.

"The soils of the Riverbank series are important agricultural soils as they mainly are located in the most densely settled areas of the St. John River Valley."

In the spring of 1950 the treatments listed below were applied on 1/200 acre plots before beginning of growth. Three rates of nitrogen, four rates of phosphorus and three rates of potassium were applied in all combinations to form a 3 x 4 x 3 factorial experiment. Two replications were used, each replication being subdivided into three sub-blocks in such a manner that a portion of the NK and NPK interactions were confounded with sub-blocks. This design and statistical analysis were developed by Li (14).

TABLE 2. — 1951 SUMMARY OF YIELD INCREASES CONTRIBUTED BY VARIOUS RATES OF N, P AND K
(Pounds of Dry Matter per Acre)

| | Date cut | | | | | | |
|---|---|---|---|---|---|---|---|
| | May 31 | June 18 | July 3 | July 27 | Aug. 24 | Oct. 5 | Total for season |
| $N_1 - N_0$ | 107.7** | 100.5** | 13.4 | −8.5 | −31.4 | −6.4 | 175.2** |
| $N_2 - N_1$ | 10.4 | 64.5** | −3.8 | 33.4 | 10.9 | −2.1 | 113.3 |
| $P_1 - P_0$ | 181.6** | 165.0** | 64.9* | 125.0** | 65.3** | 32.1** | 634.9** |
| $P_2 - P_1$ | 44.5 | 78.1** | 41.9 | 45.1 | 68.5** | 17.6 | 295.6** |
| $P_3 - P_2$ | 81.5* | 23.3 | −48.9 | −0.8 | −27.1 | −6.2 | 21.8 |
| $K_1 - K_0$ | 50.3 | 54.1* | 16.9 | 31.2 | 45.7* | 16.5 | 214.6** |
| $K_2 - K_1$ | 37.9 | 1.3 | 26.5 | 67.8* | 17.9 | 17.1 | 168.6** |

*Significant at the 5 per cent level
**Significant at the 1 per cent level

The rates of each element were as follows:

$n_0$ — No nitrogen
$n_1$ — 15 pounds of nitrogen per acre
$n_2$ — 30 pounds of nitrogen per acre
$p_0$ — No phosphorus
$p_1$ — 40 pounds $P_2O_5$ per acre
$p_2$ — 80 pounds $P_2O_5$ per acre
$p_3$ — 120 pounds $P_2O_5$ per acre
$k_0$ — No potassium
$k_1$ — 30 pounds $K_2O$ per acre
$k_2$ — 60 pounds per $K_2O$ per acre

All elements were applied annually at the beginning of growth. The date of cutting was carefully regulated so as to prevent grasses from heading out. Height of cutting was 2½ inches above ground.

Botanical analyses of the sward were made in the spring and fall of 1950 and again in the spring of 1955 by the "Point Quadrat" method (13). No detailed analyses were made in intervening years.

## RESULTS

### *Dry Matter Yields*

Increases in yields brought about by each increment of the three major elements are shown in Tables 1-5.

These data show that the most pronounced increase in yields was brought about by an application of 40 pounds of $P_2O_5$ per acre. The response to this treatment was immediate and became more pronounced each year. The average increase per season was a highly significant 688 pounds of dry matter per acre. The average yield without phosphorus was 806 pounds of dry matter per acre. Thus 40 pounds of $P_2O_5$ per acre increased the average yield during the period by over 85 per cent.

Eighty pounds of $P_2O_5$ per acre increased yields over the 40-pound rate by the highly significant amount of two hundred and fifty-three pounds of dry matter per acre per year. Highly significant increases were obtained the first 3 years of the test but the increase in the final year was not significant.

The heaviest rate of phosphorus tested, 120 pounds of $P_2O_5$ per acre, was not significantly superior to the 80-pound rate in total yield for any season.

Potassium, at 30 pounds of $K_2O$ per acre, gave highly significant increases in yield each year. This treatment gave a highly significant average increase of 287 pounds of dry matter per acre.

Potassium, at 60 pounds of $K_2O$ per acre, was superior to the 30-pound rate by the highly significant average of 174 pounds of dry matter per acre per year.

Nitrogen, at 15 pounds per acre, increased the average annual yield by the highly significant amount of 199 pounds of dry matter per acre. All significant increases due to nitrogen were obtained from the first two cuttings, the latest being June 20 in 1950.

TABLE 3. — 1953 SUMMARY OF YIELD INCREASES CONTRIBUTED BY VARIOUS RATES OF N, P AND K
(Pounds of Dry Matter per Acre)

| | Date cut | | | | | |
|---|---|---|---|---|---|---|
| | June 5 | June 23 | July 14 | Aug. 17 | Oct. 1 | Total for season |
| $N_1 - N_0$ | 182.9** | 24.6 | −2.3 | −17.8 | 16.4 | 204.1* |
| $N_2 - N_1$ | 147.7** | 25.2 | −57.0* | −29.1 | −9.6 | 77.5 |
| $P_1 - P_0$ | 257.1** | 119.3** | 112.3** | 171.6** | 62.5** | 722.7** |
| $P_2 - P_1$ | 93.4** | 104.8** | 26.7 | 54.6 | 53.9** | 333.3** |
| $P_3 - P_2$ | 63.0 | −15.6 | −20.8 | 3.2 | −15.8 | 14.5 |
| $K_1 - K_0$ | 160.9** | 75.0** | 71.0** | 84.5** | 26.4 | 417.4** |
| $K_2 - K_1$ | −3.0 | 13.8 | 42.3 | 90.8** | 20.2 | 189.2* |

*Significant at the 5 per cent level
**Significant at the 1 per cent level

TABLE 4. — 1954 SUMMARY OF YIELD INCREASES CONTRIBUTED BY VARIOUS RATES OF N, P AND K
(Pounds of Dry Matter per Acre)

| | Date cut | | | | |
|---|---|---|---|---|---|
| | May 31 | June 18 | July 6 | Aug. 12 | Total for season |
| $N_1 - N_0$ | 252.3** | 57.8** | −3.3 | −39.0 | 267.9** |
| $N_2 - N_1$ | 45.2 | 2.8 | −2.4 | −19.5 | 26.0 |
| $P_1 - P_0$ | 422.3** | 197.7** | 87.1** | 271.6** | 978.6** |
| $P_2 - P_1$ | 97.5* | 28.6 | 15.0 | −29.4 | 111.8 |
| $P_3 - P_2$ | −13.5 | −5.6 | −23.3 | 45.4 | 2.9 |
| $K_1 - K_0$ | 94.3** | 55.7** | 51.4* | 144.8** | 346.2** |
| $K_2 - K_1$ | 40.6 | 40.0 | 33.9 | 93.3** | 207.8** |

* Significant at the 5 per cent level
** Significant at the 1 per cent level

TABLE 5. — SUMMARY SHOWING AVERAGE INCREASES IN YIELD FROM VARIOUS LEVELS OF N, P AND K FOR THE YEARS 1950, 1951, 1953 AND 1954
(Pounds of Dry Matter per Acre)

| Treatments | Average increase in total yield per season |
|---|---|
| $N_1 - N_0$ | 198.8** |
| $N_2 - N_1$ | 93.2* |
| $P_1 - P_0$ | 688.3** |
| $P_2 - P_1$ | 252.9** |
| $P_3 - P_2$ | 29.8 |
| $K_1 - K_0$ | 287.0** |
| $K_2 - K_1$ | 174.2** |

The 30 pounds of nitrogen per acre was superior to the 15-pound rate by the significant average of 93 pounds for the 4-year period. In only 1 of the 4 years, namely in 1950, was the increase significant. The effect of the 30-pound rate on the distribution of growth was similar to the 15-pound rate, namely, it did not increase yields after June 20.

The total increase in yields per year resulting from an application of 30 pounds nitrogen, 80 pounds of $P_2O_5$ and 60 pounds of $K_2O$ was 1,694 pounds of dry matter. The average yield from the nil treatment was 581 pounds of dry matter per acre. Thus, if there was no interaction, the yield from the plots with the complete treatment should be in the vicinity of 2,275 pounds of dry matter per acre. The actual average yield from this treatment was 2,790 pounds per acre, indicating a positive interaction among some of the three elements. The analysis of variance (not shown here) indicated a positive and significant interaction between phosphorus and potassium only.

*Botanical Composition*

Botanical analyses for the fall of 1950 and for the spring of 1955 are given in Tables 6-7. A botanical analysis in the spring of 1950 indicated the experimental area had a reasonably uniform sward containing less than 50 per cent useful species made up of 37.0 per cent grasses and 11.2 per cent clover. In addition, there were 22.0 per cent weeds, 19.7 per cent moss, and 10.0 per cent bare ground. By the fall of the same year, 40 pounds of $P_2O_5$ had brought about definite changes in botanical composition. Compared to plots that received no phosphorus the plots receiving the 40 pounds of $P_2O_5$ contained 11.3 per cent more wild white clover, 4.8 per cent more grasses, 9.0 less weeds and 7.1 per cent less bare ground and moss. Useful species had been increased by 16.1 per cent. Higher rates of $P_2O_5$ had little additional influence on the composition of the sward.

Fifteen pounds of nitrogen per acre caused relatively slight changes in species, the most marked being a decrease of 3.5 per cent wild white clover and an increase of 5.8 per cent in grasses compared to where nitrogen was omitted. Thirty pounds of nitrogen per acre reduced clover by an additional 2.7 per cent and increased grasses by 5.0 per cent compared to the 15-pound rate.

Potassium had relatively little effect on the botanical composition by the fall of 1950.

The botanical analysis in the spring of 1955, after 5 years of treatment, showed that a general decrease in wild white clover had taken place, balanced by a general increase in grasses. Potassium had become slightly more efficient in maintaining clover than phosphorus, while the effect of nitrogen was practically unchanged.

Forty pounds of $P_2O_5$ per acre increased clover by 4.4 per cent and all grasses by 13.7 per cent compared to the zero rate. It caused a corresponding decrease of 14.9 per cent weeds and 5.4 per cent moss and increased bare ground by 2.0 per cent. Phosphorus was the element responsible for the greatest increase in useful species and correspondingly decreased weeds and moss more than the other two elements. Increasing rates of phosphorus had relatively minor effect on the percentage of species compared to the first 40-pound increment.

Thirty pounds of $K_2O$ per acre increased wild white clover by 6.2 per cent and caused a corresponding decrease of 3.8 per cent weeds and 2.9 per cent moss. It had practically no effect on grasses. Increasing $K_2O$ to 60 pounds per acre had little effect on the percentage of species compared to the 30-pound rate.

The effect of nitrogen on species remained materially unchanged from 1950. For instance, 15 pounds of nitrogen per acre decreased wild white clover by 3.3 per cent and increased all grasses by 4.5 per cent compared to the zero rate. It decreased moss by 1.5 per cent but had no effect on weeds and bare ground. Increasing nitrogen from 15 to 30 pounds per acre decreased clover by an additional 3.5 per cent and increased all grasses by 7.1 per cent compared to the 15-pound rate. It also decreased weeds, moss and bare ground by a total of 3.5 per cent. Thus, nitrogen was relatively inefficient in increasing useful species on this pasture. It brought about an increase in grasses largely at the expense of clover. Where 30 pounds of nitrogen was used the percentage of clover in the stand was 7.8 per cent, compared to 14.6 per cent where no nitrogen was used.

## DISCUSSIONS AND CONCLUSIONS

Of the three elements studied, phosphorus was the most efficient in increasing yields and improving the vegetation of this permanent pasture. The yield response to phosphorus was immediate and consistent throughout each season. During the first 3 years annual applications of 80 pounds of $P_2O_5$ per acre were required to give near maximum yields. By the fourth year the 80-pound rate failed to be superior to the 40-pound rate, indicating an accumulation of this element was taking place and the higher

TABLE 6. — EFFECTS OF VARIOUS INCREMENTS OF N, P AND K ON BOTANICAL COMPOSITION OF PASTURE SWARD
Point Quadrat Readings, Fall 1950

| Treatment per acre | Kentucky Blue Grass | Brown Top | Other grasses | Wild White Clover | Weeds | Moss | Bare Ground |
|---|---|---|---|---|---|---|---|
| | % | % | % | % | % | % | % |
| No Nitrogen | 8.6 | 32.5 | 5.6 | 23.8 | 20.5 | 3.0 | 6.0 |
| 15 lb. Nitrogen | 10.2 | 36.2 | 4.6 | 20.3 | 19.3 | 3.6 | 5.7 |
| 30 lb. Nitrogen | 10.8 | 41.2 | 4.8 | 17.6 | 17.4 | 2.2 | 6.0 |
| No Phosphorus | 7.8 | 34.0 | 6.1 | 11.6 | 25.4 | 5.6 | 9.5 |
| 40 lb. $P_2O_5$ | 10.0 | 37.4 | 5.3 | 22.9 | 16.4 | 3.2 | 4.8 |
| 80 lb. $P_2O_5$ | 9.0 | 37.1 | 4.2 | 24.7 | 18.4 | 1.6 | 5.0 |
| 120 lb. $P_2O_5$ | 12.5 | 38.2 | 4.4 | 23.1 | 16.0 | 1.4 | 4.4 |
| No Potash | 9.0 | 37.5 | 4.4 | 19.8 | 19.9 | 3.7 | 5.7 |
| 30 lb. $K_2O$ | 9.5 | 36.8 | 5.4 | 22.0 | 17.6 | 2.9 | 5.8 |
| 60 lb. $K_2O$ | 11.0 | 35.7 | 5.1 | 20.1 | 19.6 | 2.1 | 6.3 |
| Average | 9.8 | 36.7 | 5.0 | 20.6 | 19.1 | 2.9 | 5.9 |

TABLE 7. — EFFECT OF VARIOUS INCREMENTS OF N, P AND K ON BOTANICAL COMPOSITION OF PASTURE SWARD
Point Quadrat Readings, Spring 1955

| Treatment per acre | Kentucky Blue Grass | Brown Top | Other grasses | Wild White Clover | Weeds | Moss | Bare ground |
|---|---|---|---|---|---|---|---|
| | % | % | % | % | % | % | % |
| No Nitrogen | 9.5 | 37.9 | 7.0 | 14.6 | 16.2 | 4.1 | 10.7 |
| 15 lb. Nitrogen | 13.3 | 39.0 | 6.6 | 11.3 | 16.8 | 2.6 | 10.3 |
| 30 lb. Nitrogen | 13.3 | 44.5 | 8.2 | 7.8 | 14.7 | 2.0 | 9.5 |
| No Phosphorus | 6.0 | 36.3 | 6.6 | 8.4 | 25.8 | 8.1 | 8.8 |
| 40 lb. $P_2O_5$ | 14.0 | 41.8 | 6.8 | 12.8 | 10.9 | 2.7 | 10.8 |
| 80 lb. $P_2O_5$ | 12.4 | 43.8 | 8.2 | 11.0 | 13.8 | 0.6 | 10.2 |
| 120 lb. $P_2O_5$ | 15.7 | 39.9 | 7.5 | 12.8 | 13.2 | 0.2 | 10.7 |
| No Potash | 11.0 | 40.4 | 8.0 | 6.6 | 18.9 | 4.9 | 10.2 |
| 30 lb. $K_2O$ | 12.4 | 40.3 | 6.7 | 12.8 | 15.1 | 2.0 | 10.6 |
| 60 lb. $K_2O$ | 12.7 | 40.6 | 7.2 | 14.4 | 13.7 | 1.9 | 9.5 |
| Average | 12.0 | 40.5 | 7.3 | 11.3 | 15.9 | 2.9 | 10.1 |

rate was no longer necessary. On pastures previously fertilized with phosphorus the 40 pounds of $P_2O_5$ per acre would likely be sufficient but, in the initial stages of improving a pasture which is low in fertility, the 80-pound rate would be more satisfactory.

Next to phosphorus, potassium was the element most needed to improve yields and botanical composition. The effect of potassium became somewhat more pronounced as time progressed. Thirty pounds of $K_2O$ per acre increased total yields each year and increased yields for most cuttings. Sixty pounds of $K_2O$ per acre increased total yields over the 30-pound rate but only for one or two cuttings each season. However, each increase from the 60-pound rate was secured in late July or early August. This extra yield obtained at a time when it is most needed may well justify the higher rate of application. Potassium also helped more than the other elements in maintaining wild white clover, although the immediate effect it had on wild white clover was not as pronounced as with phosphorus.

Nitrogen, at 15 pounds per acre, increased yields each year. Thirty pounds per acre was superior in total yield to the 15-pound rate the first year only. All significant increases in yield due to nitrogen were secured from the first two cuttings. For instance, no significant increases from nitrogen were obtained after June 20. In one case a significant decrease in yield was obtained in July from the heavier rate of nitrogen. Of 28 cuttings taken after June 20, nitrogen was responsible for decreases in yield in 17 cases and for slight increases in remaining cases. The decreases were not significant, except in one case, but should be given some consideration due to the importance of maintaining maximum yields for this period.

In actual practice the increases obtained during June from a spring application of nitrogen are not of great importance because pastures well fertilized with phosphorus and potassium usually produce a surplus of vegetation at this time. On the other hand, nitrogen increased early grazing as the 15-pound rate always increased yields of the first cutting. Therefore, if early grazing is required, nitrogen would be beneficial but it seems likely that it would be best restricted to a portion of the pasture.

Considering above results it appears that, in areas with soil and climatic conditions similar to those that exist at the Fredericton Research Station, previously unfertilized pasture would respond well to applications of 80 pounds of $P_2O_5$ and 60 pounds of $K_2O$ per acre annually. After a period of 4 to 5 years or on pasture previously fertilized it appears that the application of $P_2O_5$ could be reduced to 40 pounds per acre. Spring applications of nitrogen could well be omitted from the major areas of pasture but could be used on relatively small, well drained areas where early grazing is desired.

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# L'ENRACINEMENT DES BOUTURES D'ORME D'AMERIQUE (*ULMUS AMERICANA* L.) SOUS UN FAIBLE ECLAIREMENT

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## SOMMAIRE

La principale difficulté rencontrée dans la multiplication de l'orme d'Amérique par boutures de rameaux feuillés fut la flétrissure prématurée du feuillage, empêchant tout enracinement subséquent. La réduction de l'éclairement au cours de la période d'enracinement s'avéra un moyen satisfaisant de remédier à ce trouble. Un éclairement d'environ 1200 lux donna généralement de bons résultats. On croit que la réduction de l'éclairement produit une diminution de la transpiration des boutures et favorise ainsi l'enracinement.

Trois facteurs principaux ont semblé affecter l'effet de l'éclairement: 1) L'époque du prélèvement au cours de la saison: un degré d'éclairement un peu plus élevé s'avéra préférable à la fin de l'été. 2) Les conditions atmosphériques durant la saison de prélèvement: des boutures prélevées durant une année pluvieuse et contenant un haut pourcentage d'humidité racinèrent bien même sous un éclairement inférieur à 1200 lux. 3) L'âge des arbres-mères: les boutures provenant d'ormes plus agés ont semblé réagir plus au degré d'éclairement que celles provenant de tout jeunes arbres.

## INTRODUCTION

Le travail accompli jusqu'en 1949 sur la multiplication de l'orme par boutures fut très minime. Depuis, les recherches en vue de découvrir une lignée d'orme d'Amérique résistante à la maladie hollandaise ont contribué à intensifier le développement d'une méthode de multiplication par boutures de rameaux feuillés. Utilisant des jets-brouillards maintenus en opération au-dessus des boutures, quelques auteurs ont rapporté des résultats bons dans le cas de Doran (6), variables dans le cas de Bretz (3) et celui de Bretz et Swingle (4).

A la Ferme Expérimentale de L'Assomption ainsi qu'à d'autres centres de recherches, la principale difficulté que présenta l'enracinement en serre fut la flétrissure du feuillage empêchant tout enracinement subséquent. Selon Dimond *et al.* (5), l'orme d'Amérique est très susceptible à la flétrissure physiologique, trouble qui serait dû à la formation de bulles d'air dans les vaisseaux du xylem, réduisant la montée de l'eau dans ces derniers. Bretz (3) affirme que le seul moyen qu'il ait découvert pour conserver turgescent le feuillage des boutures consista dans l'usage d'un système de jets-brouillards; sinon les feuilles se fanaient, même lorsque l'humidité relative du milieu ambiant approchait le point de saturation.

Pour réduire cette flétrissure du feuillage, on a imaginé, à L'Assomption, d'abaisser l'éclairement à un bas degré au cours de la période d'enracinement. On peut déduire des principes généraux énoncés par Meyer et Anderson (8) que l'un des effets d'un faible éclairement est de diminuer la transpiration sensiblement grâce à une réduction du degré et de la durée d'ouverture des stomates; il y a encore économie de la réserve d'eau des boutures par suite d'un abaissement de la température du feuillage et d'un

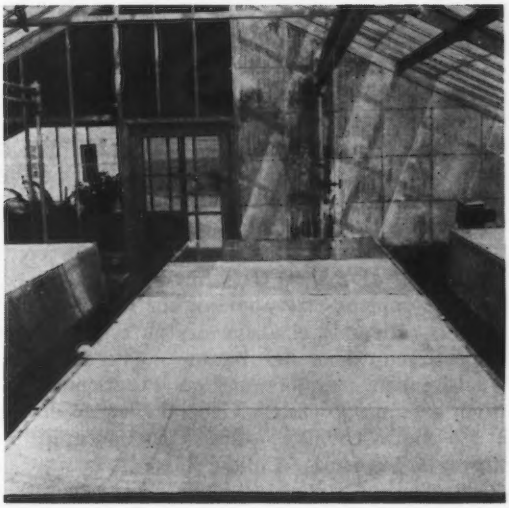

FIGURE 1. Matériaux et méthode utilisés afin d'obtenir les différents degrés d'éclairement, i.e., toit chaulé, vitres non chaulées ou chaulées à différents degrés.

ralentissement des activités vitales telles que la photosynthèse et la respiration. La multiplication végétative de plusieurs espèces de plantes, selon Bradford (2), s'est effectuée avec succès sous de faibles éclairements allant de 200 à 100 bougies-pieds (1 bougie-pied = 10.8 lux) et, même en certains cas, allant aussi bas que 10 bougies-pieds. Hunter et Nelson[1] font tout de même remarquer qu'une certaine augmentation de lumière peut favoriser l'enracinement des boutures contenant moins d'eau à l'époque de leur prélèvement par suite de la sécheresse de la saison ou de l'aoûtement, et, conséquemment, étant moins exposées à souffrir d'un excès de transpiration. Selon Kains et McQuesten (7), il faut donner aux boutures toute la lumière qu'elles peuvent supporter sans inconvénient, puisque la lumière est nécessaire à l'activité photosynthétique du feuillage et au bon développement des racines.

Le but de cet article est donc de montrer l'influence favorable d'un faible éclairement sur l'enracinement des boutures de rameaux feuillés d'orme d'Amérique.

## MATERIEL ET METHODE

Les expériences ont été effectuées en serre à la Ferme Expérimentale de L'Assomption au cours des années 1951, 1952, 1953 et 1958. Elles portèrent sur l'enracinement de boutures de rameaux feuillés d'orme d'Amérique prélevées sur la pousse de l'année et d'une longueur moyenne de 3.75 pouces. Avant la plantation, la base des boutures était trempée dans une solution de 50 mg. d'acide indolbutyrique par litre d'eau durant une période de 24 heures. Le sable, utilisé comme milieu d'enracinement, était de texture moyenne, le pH de 5.90 et le pourcentage de matière colloidale (limon et argile) de 9 pour cent.

[1]Hunter, A. W. S., et S. H. Nelson, Ferme Expérimentale Centrale, Ottawa. *Correspondance personnelle.*

L'humidité relative et la température à l'intérieur de la serre varièrent respectivement de 80 pour cent à 100 pour cent et de 70°F. à 80°F., excepté en 1958 où la température a été un peu plus haute. On maintint l'humidité élevée grâce à un fort chaulage de la serre et à un système d'humidification spécial. Ce dernier, en opération à tous les ans excepté en 1951, comportait des jets de marque Monarch no. 3.00, 120°W, installés dans les allées de la serre à six pouces au-dessus du plancher et maintenus en opération lorsque nécessaire, principalement au cours des après-midis ensoleillées. Comme les boutures étaient plantées sur des tables disposées à 3 pieds au-dessus du plancher, elles n'étaient pas humectées directement par l'eau de ces jets-brouillards. Deux ou trois fois par jour, on vaporisait les boutures et un arrosage copieux était appliqué sur le plancher et les cotés de la serre.

En variant le chaulage des vitres de la serre ou d'une surface vitrée disposée au-dessus des boutures (Figure 1), on obtint les différences d'éclairement requises dans ces expériences. Les lectures de lumière se firent avec un posemètre General Electric du type DW-58, 3 fois par jour et à 6 pouces environ au-dessus des boutures. En 1958, ces lectures furent prises directement en unité d'éclairement, c'est-à-dire, en bougies-pieds (foot-candles) grâce à des écrans spéciaux fixés à l'instrument. De 1951 à 1953, faute de ces écrans, on établit le degré de lumière en unité de brillance (lamberts-pieds). Il n'éxiste pas de facteur précis pour convertir le lambert-pied en bougies-pieds. A la suite d'essais comparatifs faits à peu près dans les mêmes conditions d'environnement, on a tout de même jugé que le fait de réduire de 25 pour cent les données obtenues en lamberts-pieds donnait une bonne idée du degré d'éclairement exprimé en bougies-pieds. Dans cet article, le degré d'éclairement est toujours indiqué en lux.

Vu que les résultats d'enracinement sont exprimés sous forme de pourcentage et que certains sont très pauvres, les données consignées dans les tableaux 3 et 4 ont été converties en valeur angulaire avant d'être soumises à l'analyse statistique. Au tableau 5, comme la dispersion des données est modérée, les valeurs absolues ont été utilisées pour l'analyse des résultats.

## RESULTATS EXPERIMENTAUX

Avant de présenter les résultats obtenus, il est bon de donner une idée des conditions d'humidité atmosphérique qui ont prévalu à chaque saison

TABLEAU 1.—EXCÉDENT DE L'ÉVAPORATION SUR LA PRÉCIPITATION EN POUCES

| Année | Juin | Juillet | Août | Total |
|---|---|---|---|---|
| 1951 | 2.3 | 3.6 | 0.6 | 6.5 |
| 1952 | 4.9 | 3.9 | 0.0 | 8.8 |
| 1953 | 5.1 | 4.5 | 8.0 | 17.6 |
| 1958 | 2.7 | 1.1 | 1.3 | 5.1 |
| Moyenne de 1951-1958 incl. | 3.1 | 3.6 | 2.8 | 9.5 |

TABLEAU 2.—POURCENTAGES D'ENRACINEMENT DE BOUTURES D'ORME D'AMÉRIQUE PRÉLEVÉES SUR DES ARBRES DE 8 ANS EN 1951 ET RECOUVERTES D'UNE TOILE APRÈS LEUR PLANTATION. (100 BOUTURES PLANTÉES À CHAQUE DATE)

| Date de plantation | Boutures enracinées |
|---|---|
| | (%) |
| 22 juin | 80 |
| 29 juin | 90 |
| 6 juillet | 40 |
| 13 juillet | 40 |
| 20 juillet | 64 |
| 27 juillet | 79 |
| 11 août | 3 |
| 18 août | 8 |
| 25 août | 37 |
| 1 septembre | 0 |
| 15 septembre | 0 |
| 21 septembre | 0 |

où des expériences ont été faites. Ce facteur influence beaucoup le contenu en eau des boutures et conséquemment leur enracinement. L'excédent de l'évaporation sur la précipitation étant d'autant plus grand que l'année est plus sèche, on peut déduire des données consignées au tableau 1 que les saisons de 1951 et 1958 furent humides, celle de 1952, moins humide, et celle de 1953, sèche.

*Résultats de 1951*

Les expériences de cette année démontrèrent principalement que par saison humide les boutures d'orme pouvaient raciner sous un très faible éclairement, particulièrement lorsqu'elles étaient prélevées en juin et juillet.

Des groupes de boutures furent prélevées et plantées à chaque semaine du 22 juin au 21 septembre. Malgré un fort chaulage de la serre, il fut impossible de garder le feuillage turgescent sans l'usage d'une épaisse toile (toile à moissonneuse). Celle-ci demeurait continuellement au-dessus des boutures durant les 10 premiers jours après la plantation; par la suite, elle était enlevée quelques heures durant le jour. Sous la toile, l'éclairement variait de 10 à 100 lux, alors que sans toile il pouvait être de 2000 lux. Les boutures plantées du 22 juin au 27 juillet racinèrent de façon satisfaisante; par contre, celles qui furent plantées plus tard racinèrent ordinairement mal ou pas du tout (tableau 2).

L'exposition des boutures à un faible éclairement s'avéra surtout importante au cours des deux premières semaines après la plantation. Deux groupes de boutures prélevées le 18 juillet et recouverts d'une toile après la plantation, l'un durant une semaine et l'autre durant deux semaines, racinèrent respectivement dans la proportion de 32 et 70 pour cent.

En fin de saison, un degré d'éclairement encore faible, mais plus élevé que précédemment, s'avéra profitable. Des boutures prélevées le 29 août sur de jeunes ormes de 3 ans furent plantées, un groupe sous toile (10 à 100 lux) et l'autre sous un double de coton à fromage (1500 lux). Les résultats d'enracinement furent les suivants: 8 pour cent sous la toile et 60 pour cent sous le coton à fromage.

TABLEAU 3.—POURCENTAGES D'ENRACINEMENT DE BOUTURES D'ORME D'AMÉRIQUE PRÉLEVÉES LES 14 JUILLET ET 4 AOÛT SUR DES ARBRES D'ÂGES DIFFÉRENTS ET EXPOSÉES À DIFFÉRENTS DEGRÉS D'ÉCLAIREMENT EN 1953

| 14 juillet | | | | | 4 août | | | | | Enracinement moyen | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Eclairement | Température | Enracinement % Âge des arbres-mères | | | Eclairement | Température | Enracinement % Âge des arbres-mères | | | % | Conversion angulaire* |
| Lux | °F. | 2 ans | 8 ans | 15 ans | Lux | °F. | 2 ans | 8 ans | 15 ans | | |
| 6,793 | 80 | 100 | 64 | 26 | 5,303 | 76 | 60 | 22 | 32 | 50.8 | 45.5 |
| 6,080 | 79 | 94 | 70 | 40 | 4,644 | 75 | 80 | 42 | 50 | 62.7 | 52.4 |
| 3,910 | 78 | 98 | 70 | 54 | 3,089 | 74 | 74 | 66 | 47 | 68.1 | 55.6 |
| 2,646 | 78 | 96 | 80 | 66 | 2,246 | 74 | 80 | 70 | 60 | 74.7 | 59.8 |
| D.R. (Différence requise) P — 0.05 | | | | | | | | | | | 10.0 |

* Vu que les données sont exprimées en pourcentage et qu'elles accusent de grandes différences, elles ont été converties en valeur angulaire avant d'être soumise à l'analyse statistique.

TABLEAU 4.—POURCENTAGES D'ENRACINEMENT DE BOUTURES D'ORME D'AMÉRIQUE PRÉLEVÉES LES 19 JUIN ET 4 JUILLET 1958 D'ARBRES D'ÂGES DIFFÉRENTS ET EXPOSÉES À DIFFÉRENTS DEGRÉS D'ÉCLAIREMENT

| 19 juin | | | | 4 juillet | | | | Enracinement moyen | |
|---|---|---|---|---|---|---|---|---|---|
| Eclairement | Température | Enracinement % Âge des arbres-mères | | Eclairement | Température | Enracinement % Âge des arbres-mères | | % | Conversion angulaire* |
| Lux | °F. | 15 ans | 50 ans | Lux | °F. | 15 ans | 50 ans | | |
| 12,691 | — | 35 | 2 | 11,647 | 85 | 18 | 1 | 14.0 | 22.0 |
| 8,159 | — | 46 | 5 | 7,675 | 80 | 22 | 4 | 19.2 | 26.0 |
| 5,554 | — | 60 | 12 | 4,865 | 79 | 38 | 4 | 28.0 | 31.9 |
| 1,109 | — | 71 | 21 | 1,216 | 77 | 52 | 13 | 39.2 | 38.8 |
| D.R. P — 0.05 | | | | | | | | | 5.4 |
| D.R. P — 0.01 | | | | | | | | | 7.5 |

* Vu que les données sont exprimées en pourcentage et que certaines sont très basses, elles ont été converties en valeur angulaire avant d'être soumises à l'analyse statistique.

*Résultats de 1952*

Ce fut une saison moins humide que celle de 1951 (tableau 1). Parce que contenant probablement moins d'eau qu'en 1951 et peut-être moins exposées à un excès de transpiration, les boutures profitèrent d'une augmentation du degré d'éclairement qui, tout-de-même, restait encore bas.

Notons que l'emploi du coton à fromage comme matériau de couverture en 1952 et les années subséquentes rendit l'usage des jets-brouillards (à proximité du plancher) nécessaire pour obtenir un degré d'humidité relative élevé dans l'environnement immédiat des boutures.

Deux groupes de boutures furent plantés l'un sous un double de coton à fromage (1500 lux) durant toute la période d'enracinement et l'autre sous une toile (10 à 100 lux) durant les deux premières semaines suivant la plantation. Répétée à deux dates, soit les 13 juin et 4 juillet, l'expérience donna un enracinement moyen de 64 pour cent dans le cas du coton à fromage et de 8 pour cent dans le cas de la toile.

D'autres groupes de boutures plantées hebdomadairement du 23 juin à la fin de juillet et recouvertes d'un double de coton à fromage (1500 lux) durant toute la période d'enracinement racinèrent dans la même proportion (70 pour cent) que celles plantées en 1951 et recouvertes d'une épaisse toile (10 à 100 lux) durant la période indiquée précédemment pour cette année là.

*Résultats de 1953*

Cette saison fut sèche. Un éclairement plus fort qu'en 1951 mais encore relativement faible donna les meilleurs résultats.

Le 14 juillet, on fit une expérience qui fut répétée le 4 août. Elle comportait quatre traitements ou éclairements différents, deux réplications à chaque date, et 75 boutures par parcelle, ces dernières provenant en nombre égal d'arbres de 2, 8 et 15 ans. Afin d'assurer les différences requises entre les degrés d'éclairement, une toile séparait les boutures soumises aux divers traitements. La température dans le voisinage immédiat des boutures varia peu de l'éclairement le plus fort au plus faible (tableau 3). Il y eut une tendance très marquée de l'enracinement à augmenter par suite d'une diminution de l'éclairement et l'analyse statistique indiqua une différence significative en faveur d'un faible éclairement (tableau 3). Même si l'analyse des données n'a pas montré d'intéraction significative entre l'éclairement et l'âge des arbres-mères, les arbres plus agées ont semblé réagir plus aux variations d'éclairement que les plus jeunes.

*Résultats de 1958*

La saison de 1958 a été humide (tableau 1). En juin et juillet, le plus faible éclairement à l'essai donna les meilleurs résultats; plus-tard, les boutures bénéficièrent d'un accroissement modéré de lumière.

Quatre expériences comportant quatre traitements de lumière et quatre réplications furent effectuées respectivement les 19 juin, 4 juillet, 31 juillet, et 14 août. Le plan expérimental fut celui du carré latin avec parcelles divisées pour des groupes de boutures venant d'arbres-mères d'âge différents, soit 15 et 50 ans dans les deux premières expériences, et 4 et 8 ans dans les deux dernières. Des vitres chaulées à différents degrés et posées à 1 pied au-dessus des boutures permirent d'obtenir les degrés d'éclairement

désirés. L'homogénéité respective des deux premières et des deux dernières expériences ayant été confirmée par la formule de Bartlett, on fit une analyse de variance combinée de chacun de ces deux groupes d'expériences. Les tableaux 4 et 5 montrent les conditions d'environnement sous lesquelles ces expériences ont été effectuées ainsi que les résultats obtenus. Comme un nouveau chaulage fut appliqué à la serre le 23 juillet, il ne faut pas s'étonner que l'éclairement fut beaucoup plut fort dans les deux premières expériences (tableau 4) que dans les deux dernières (tableau 5). Les données de température et d'humidité représentent les moyennes du jour seulement, et n'incluent pas la période nocturne. Les chiffres consignés au tableau 6 représentent la diminution de l'eau du 19 juin au 3 juillet 1958 dans des groupes de boutures d'orme prélevées sur quatre arbres.

Le tableau 4 indique une augmentation constante de l'enracinement par suite d'une diminution de l'éclairement pour les boutures plantées les 19 juin (figures 2 et 3) et 4 juillet. L'analyse de la variance a montré que le traitement 3 fut décidément meilleur que les deux premiers et que le traitement 4 a été supérieur à tous les autres. Notons que ce dernier traitement ne comportait qu'un éclairement de 1100 à 1200 lux.

Par contre avec des boutures plantées les 31 juillet et 14 août, le plus faible éclairement a donné un pourcentage d'enracinement plus bas que ceux obtenus avec les trois autres traitements de lumière (tableau 5). En fait, l'analyse de la variance a révélé une différence significative entre le dernier traitement d'une part et les deuxième et troisième traitements d'autre part. Comparant les résultats individuels des deux expériences incluses dans le tableau 5, on note qu'un accroissement de lumière s'est avéré favorable avec l'avancement de la saison; le traitement 3 donna le meilleur pourcentage d'enracinement pour les boutures plantées le 31 juillet, tandis que ce fut le traitement 2 pour celles plantées le 14 août (figure 4 et 5).

## DISCUSSION ET CONCLUSION

1. L'exposition à un faible éclairement au cours de la période d'enracinement a favorisé la formation des racines chez les boutures de rameaux feuillés d'orme d'Amérique (tableaux 2, 3, 4 et 5). En plusieurs cas, comme pour les boutures plantées les 14 juillet et 4 août 1953 ainsi que les 19 juin et 4 juillet 1958, les coefficients de corrélation (allant de $-0.683$ à $-0.954$) entre l'enracinement et le degré d'éclairement ont confirmé cette assertion.

Déja précédemment, cette relation entre l'abaissement de l'éclairement et l'augmentation de l'enracinement a été imputée à une diminution de la transpiration chez les boutures, ce qui empêcha ou ralentit la flétrissure du feuillage. Le fait que la plupart des boutures non encore enracinées une quarantaine de jours après leur plantation aient perdu leurs feuilles prématurément confirme cette hypothèse. C'est d'ailleurs dans les traitements comportant les plus faibles degrés d'éclairement qu'on a compté le plus de boutures possédant encore leur feuillage parmi celles qui n'étaient pas enracinées à cette époque.

2. Même s'il fut toujours relativement faible, le degré d'éclairement qui a donné le meilleur pourcentage d'enracinement a varié au cours d'une même saison et d'une année à l'autre.

FIGURE 2.

FIGURE 3.

FIGURES 2 ET 3. Enracinement de boutures prélevées le 19 juin 1958 sur des arbres-mères de 15 ans (figure 2) et 50 ans (figure 3), et exposées aux taux d'éclairement suivants exprimés en lux: 12691 (1), 8159 (2), 5554 (3), et 1109 (4).

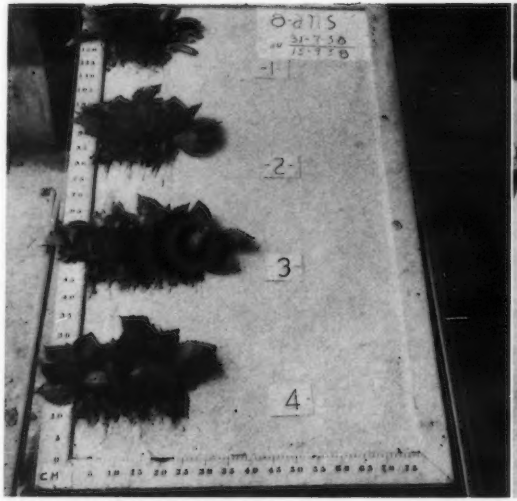


FIGURE 4.

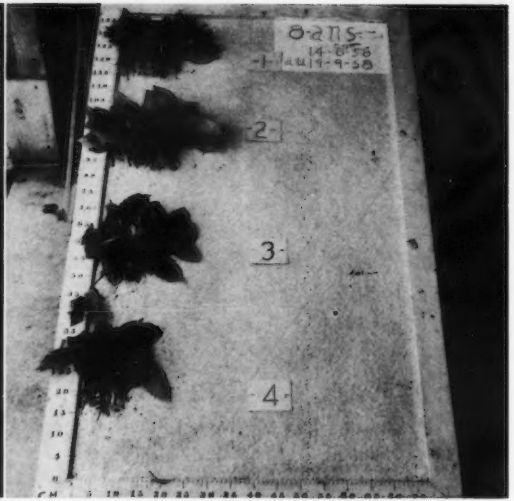


FIGURE 5.

FIGURES 4 ET 5. Enracinement de boutures plantées les 31 juillet (figure 4) et 14 août (figure 5), 1958. Les taux d'éclairement exprimés en lux ont été les suivants: 5899 (1), 4155 (2), 2034 (3), et 797 (4) pour les boutures de la figure 4, et 4821 (1), 3488 (2), 1701 (3) et 635 (4) pour celles de la figure 5. Notons qu'un degré d'éclairement plus élevé, mais encore faible, a semblé favoriser l'enracinement avec l'avancement de la saison.

TABLEAU 5.—POURCENTAGES D'ENRACINEMENT DE BOUTURES D'ORME D'AMÉRIQUE PRÉLEVÉES LES 31 JUILLET ET 14 AOÛT 1958 SUR DES ARBRES D'ÂGES DIFFÉRENTS ET EXPOSÉES À DIFFÉRENTS DEGRÉS D'ÉCLAIREMENT

| 31 juillet | | | | | 14 août | | | | | Enracinement moyen | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Eclairement | Température | Humidité relative | Enracinement % | | Eclairement | Température | Humidité relative | Enracinement % | | % | Valeur absolue* |
| | | | Age des arbres-mères | | | | | Age des arbres-mères | | | |
| Lux | °F. | % | 4 ans | 8 ans | Lux | °F. | % | 4 ans | 8 ans | | max: 25 |
| 5,899 | 75 | 88 | 90 | 50 | 4,821 | 71 | 93 | 70 | 32 | 60.5 | 15.1 |
| 4,155 | 74 | 90 | 87 | 51 | 3,488 | 70 | 93 | 73 | 42 | 63.2 | 15.8 |
| 2,034 | 73 | 90 | 92 | 78 | 1,701 | 68 | 93 | 70 | 28 | 67.0 | 16.8 |
| 797 | 73 | 90 | 92 | 48 | 635 | 68 | 94 | 58 | 22 | 55.0 | 13.8 |
| D.R.P.—0.05 | | | | | | | | | | | 2.0 |

* Comme les données sont exprimées en pourcentage et que leur dispersion est modérée, les valeurs absolues ont été utilisées pour l'analyse statistique.

Le degré d'éclairement le plus favorable augmenta ordinairement du début à la fin de la saison. En juin et juillet 1951, un éclairement de 10 à 100 lux donna d'excellents résultats, alors qu'en août de la même année un éclairement de 1500 lux s'avéra préférable. Un degré d'éclairement de 1000 à 1200 lux se montra très satisfaisant en juin et juillet 1958, tandis que l'éclairement optimum varia de 2000 à 3500 lux en août. Comme le contenu en eau décroît avec l'avancement de la saison par suite de l'aoûtement, il semble que les boutures prélevées tardivement sont moins exposées à un excès de transpiration au cours de la période d'enracinement et qu'une certaine augmentation de lumière loin de les faire se faner favorise leur enracinement.

D'une année à l'autre, le degré d'éclairement optimum peut varier, étant plus bas par année humide que par année sèche.

3. Même si l'analyse statistique n'a pas indiqué de différence significative, l'influence du degré d'éclairement a semblé plus importante dans le cas des boutures venant d'ormes de 8 ans (et plus) que dans le cas de boutures prélevées sur des arbres de 2 et 4 ans (tableaux 3 et 5). Il peut se faire que ces dernières, racinant assez facilement et rapidement, soient moins affectées par l'accroissement de transpiration causé par un éclairement plus fort.

On nota toujours des différences significatives entre les pourcentages d'enracinement de boutures prélevées sur des arbres-mères d'âges différents, les boutures racinant d'autant mieux que les arbres-mères étaient plus jeunes (tableaux 3, 4 et 5). Ce fait, d'après Blair *et al.* (1), serait dû aux phases de croissance plus ou moins avancées de la plante-mère. Ces phases sont de nature physiologique et, bien que leurs causes fondamentales ne soient pas complètement connues, leurs caractères externes sont bien définis et elles sont généralement désignées comme phases juvénile, transitoire, adulte et sénile.

4. La date du prélèvement des boutures, pourvu que ce soit en juin et juillet, n'a pas semblé affecter l'influence du facteur lumière sur l'enracinement. Plus tard dans la saison comme pour les expériences effectuées les 31 juillet et 14 août 1959, l'analyse de la variance indiqua une intéraction significative entre la date du prélèvement des boutures et l'influence du degré d'éclairement sur l'enracinement, les boutures profitant d'une augmentation de l'éclairement avec l'avancement de la saison. (tableau 5)

5. L'influence favorable d'un faible éclairement sur l'enracinement des boutures d'orme d'Amérique semble être directe. On ne peut l'attribuer, du moins pour une part importante, aux variations de température et d'humidité relative qui auraient pu être causées par les variations de lumière.

En plusieurs cas, l'influence de l'éclairement sur l'enracinement a été significative et pourtant les variations de température entre les divers traitements ont été très minimes (tableaux 3 et 5). Quant à l'humidité relative, elle a été maintenue à peu près uniforme (tableau 5), grâce à des arrosages assez fréquents et à des jets-brouillards en opération près du plancher de la serre.

TABLEAU 6.—POURCENTAGE D'EAU DES BOUTURES PAR RAPPORT AU POIDS SEC, LE JOUR DE LEUR PRÉLÈVEMENT EN 1958

| Date du prélèvement des boutures | Age des arbres-mères | | | | | |
|---|---|---|---|---|---|---|
| | 15 ans | | | 50 ans | | |
| | Feuilles | Tiges | Boutures entières | Feuilles | Tiges | Boutures entières |
| 19 juin | 182 | 208 | 186 | 190 | 216 | 197 |
| 3 juillet | 165 | 167 | 166 | 174 | 187 | 177 |
| Différence | 17 | 41 | 20 | 16 | 29 | 20 |

6. Il se peut que l'abaissement de l'éclairement pour contrôler la flétrissure prématurée du feuillage des boutures avant l'enracinement soit une méthode plus pratique à L'Assomption que l'usage des jets-brouillards maintenus en opération au-dessus des boutures. Cette dernière méthode fut utilisée par Doran (6), Bretz (3), Bretz et Swingle (4).

En théorie l'usage des jets-brouillards peut s'avérer nécessaire en certaines régions où le climat est particulièrement chaud et sec, la transpiration des plantes très élevée, ou bien lorsque l'enracinement des boutures s'effectue à l'extérieur en pleine lumière. C'est une méthode qui réduit la transpiration à un niveau plus bas que par un faible éclairement, tout en permettant aux boutures de profiter d'une abondance de lumière pour l'activité photo-synthétique du feuillage.

Tout-de-même, l'usage des jets-brouillards nous semble présenter deux inconvénients: 1. Danger d'interruption accidentelle du système d'humidification, ce qui peut causer la flétrissure du feuillage en peu de temps. 2. Excès d'eau sur le feuillage, ce qui favorise le développement de taches sur les feuilles et leur chute prématurée.

A L'Assomption, où la transpiration des plantes est plutôt modérée, l'abaissement de l'éclairement s'est avéré satisfaisant pour contrôler la flétrissure du feuillage des boutures d'orme en serre et assurer un enracinement convenable, tout en éliminant les inconvénients que présente l'utilisation des jets-brouillards.

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## ABSTRACT

The main difficulty encountered in the propagation of American elm (*Ulmus Americana* L.) by softwood stem cuttings was the premature wilting of foliage, keeping them from subsequent rooting. Decreasing the natural intensities of illumination in our greenhouse during rooting proved to be a satisfactory method of controlling premature wilting. Good results were generally obtained with an illumination of about 1200 lux. It is believed that the transpiration of cuttings is decreased by the reduction of illumination, which so favours rooting. The effect of illumination has seemed to be influenced by three main factors: 1) The time of taking cuttings during summer; at the end of the summer a somewhat higher degree of illumination was advantageous. 2) Meteorological conditions during the season of taking cuttings; good rooting was still obtained within cuttings taken during very rainy summers and containing a high percentage of moisture even under an illumination lower than 1200 lux. 3) The age of parent-trees; the cuttings coming from old trees seemed to be more influenced by the degree of illumination than those coming from young ones.

# EVALUATION OF BIRDSFOOT TREFOIL

## I. Compatibility of *Lotus corniculatus* L. with other legumes and grasses[1]

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### ABSTRACT

Four tests were conducted on three soil types, at Ottawa, Ont., and Ste. Anne de la Pocatière, Que., to study the comparative behaviour of birdsfoot trefoil (*Lotus corniculatus* L.) in various mixtures. Data show that birdsfoot trefoil was more persistent than alfalfa in long-term leys on imperfectly drained soils. It also persisted longer than ladino or alsike clovers on the two soil types where these crops were compared. Data confirm previous findings that it possesses very little competitive ability. Accordingly, to reduce competition, birdsfoot trefoil should be used in simple mixtures with non-aggressive species. Timothy seemed generally to be the best companion grass. Red clover should be used with great care since it has a marked detrimental effect on its contribution to the crop. Preliminary results suggest the value of an orchardgrass-birdsfoot trefoil mixture in dry habitats.

### INTRODUCTION

It has been recognized for many years that there is a need in Eastern Canada for a legume that will withstand grazing and maintain a high level of productivity in long-term pastures. Birdsfoot trefoil (*Lotus corniculatus* L.) appears to be the most promising of the pasture crops that have been introduced during the past 25 years.

### REVIEW OF LITERATURE

Hughes and Heath (3), MacDonald (4), and Midgley (5) described birdsfoot trefoil and pointed out many of its virtues and weaknesses. MacDonald (4) and Midgley (5) state that birdsfoot trefoil is superior to alfalfa on poorly drained clay soils and better than ladino clover on drier lands, because of its deep root system. Midgley (5) also says that it will withstand more abuse, such as close grazing, than alfalfa or most of the clovers. When directly compared with ladino-timothy, he found that trefoil-timothy yielded less dry matter during the first 2 years after seeding, but yielded more dry matter during the remaining 5 years of the experiment. After 7 crop years trefoil-timothy averaged over a half-ton more dry matter per acre than ladino-timothy. Bachtell (1) found that trefoil yielded most in simple mixtures. The inclusion of red clover in a trefoil-timothy mixture increased the yield in the first harvest year but decreased it for the duration of the test, while the inclusion of ladino depressed the stand of trefoil with the consequence that the first cut of the season was high and the second cut was quite low particularly after the first 3 years. The trefoil had not become well established because of the ladino competition.

Davis and Bell (2) in grazing trials using lambs found that a ladino-bluegrass mixture gave greater animal yields the first year but trefoil-

[1]Joint publication from the Experimental Farm, Canada Department of Agriculture, Ste. Anne de la Pocatière, and the Genetics and Plant Breeding Research Institute (Contribution No. 15), Canada Department of Agriculture, Ottawa, Ont.

bluegrass gave greater yields in the last 2 years of a 3-year test. Under the intense grazing management used, trefoil was practically eliminated at the end of the 3-year period. Washko and Williams (6) reported that grass-alfalfa produced more dry matter, animal-carrying capacity and T.D.N. than birdsfoot trefoil mixtures. On imperfectly drained soils Winch (7) recommends the use of birdsfoot trefoil to replace alfalfa, ladino and even red clover in hay fields where 2 or more years of hay are desired. The Ontario Department of Agriculture (8) presents data showing that total production of dry matter for 2 years from a Viking trefoil-Climax timothy mixture was over 1,000 pounds greater than from a mixture of Lasalle red clover and Climax timothy, while the addition of Empire birdsfoot to a bluegrass pasture doubled its production.

## MATERIALS AND METHODS

Several experiments were laid out at Ottawa, Ontario, and Ste. Anne de la Pocatière, Quebec, to determine the place of birdsfoot trefoil in the pasture program of Eastern Canada and to study its compatibility with other legumes and grasses in simple and complex mixtures. The best check mixtures were chosen according to local recommendations as well as the most appropriate plot technique and management practices. Composition and rate of seeding of mixtures are given in Tables 1, 2, 4 and 6. Clipping

TABLE 1.—YIELDS IN POUNDS OF DRY MATTER PER ACRE OF THREE MIXTURES GROWN ON KAMOURASKA CLAY

| Mixture and rate of seeding in lb. per acre | 1950 | 1951 | 1952 | 1953 | 1954 | Av. |
|---|---|---|---|---|---|---|
| Alfalfa 8, Timothy 8 | 7592 | 6718 | 3442 | 2574 | 1191 | 4303 |
| Empire 4, Timothy 8 | 4902 | 5204 | 3818 | 3306 | 2374 | 3921 |
| Empire 4, Red Clover 4, Timothy 8 | 5534 | 4622 | 3924 | 3404 | 2693 | 4035 |
| L.S.D. (.05) | 894 | 1538 | 898 | 622 | 338 | 412 |

TABLE 2. — YIELDS IN POUNDS OF DRY MATTER PER ACRE OF NINE MIXTURES GROWN ON ST. ANDRÉ GRAVELLY LOAM

| Mixture and rate of seeding in lb. per acre | Yields of dry matter in lb. per acre | | | | | |
|---|---|---|---|---|---|---|
| | 1951 | 1952 | 1953 | 1954 | 1955 | Av. |
| Empire 4, Timothy 8 | 1532 | 2700 | 1144 | 3548 | 2234 | 2232 |
| Empire 4, Timothy 8, Red Clover 4 | 3090 | 3522 | 1196 | 3288 | 2279 | 2675 |
| Empire 4, Orchardgrass 10 | 2500 | 3540 | 1894 | 4020 | 2888 | 2968 |
| Empire 4, Meadow Fescue 14 | 1598 | 2460 | 1084 | 3646 | 2139 | 2185 |
| Empire 4, Red Fescue 10 | 2672 | 4166 | 2362 | 4424 | 2914 | 3308 |
| Empire 4, Ottawa 39 10 | 2314 | 3940 | 2259 | 4452 | 2581 | 3109 |
| Empire 4, Kentucky Blue Grass 5 | 2594 | 3694 | 2072 | 3776 | 2453 | 2918 |
| Empire 4, Bromegrass 15 | 2602 | 3912 | 2344 | 3782 | 2383 | 3005 |
| Alfalfa 6, Bromegrass 15 | 3632 | 5320 | 3772 | 4608 | 3546 | 4176 |
| L.S.D. (.05) | 430 | 398 | 300 | 178 | 289 | 159 |

of herbage was done with a Gravely tractor mower at a height of 1½ inches in all cases. Visual estimates of percentage contribution to yield or of ground cover of mixture components were taken according to methods described in Reference 9. A nurse crop of cereal was used at seeding and removed between heading and milk stage, except for Experiment No. 2, which was seeded without a nurse crop. In each case the design used was a randomized block. The number of replicates varied from four, in Experiments 1 and 4, to six in Experiments 2 and 3, while plot size was either 30 feet x 5 feet or 21½ feet x 5 feet. Conditions specific to each experiment are described hereafter.

*Experiment 1, Ste. Anne de la Pocatière*

This experiment was laid out in 1949 on Kamouraska clay, a very fertile soil but imperfectly drained and with a pH of 6.8. It was comprised of 16 mixtures, 14 of which included alfalfa in various combinations with bromegrass or timothy, while 2 included birdsfoot trefoil; only the trefoil and the check mixtures are reported here. Four hundred pounds per acre of 4-12-6 were applied at seeding time only. The mixtures were cut for hay in 1950; in 1951 they were cut once for hay and twice as pasture; thereafter they were clipped four times a year to simulate grazing.

*Experiment 2, Ste. Anne de la Pocatière*

This experiment was located on St. André gravelly loam, a soil naturally low in fertility, but deep and well drained with a pH value of 5.2. Nine mixtures were seeded and clipped three to four times a year to simulate grazing. In the fall of 1949, 12 tons of manure per acre were applied, followed by 300 pounds of 4-12-6 at seeding time in 1950, and 400 pounds 4-12-6 in the spring of 1953 and in the fall of 1954.

*Experiment 3, Ottawa*

Nine mixtures were seeded in 1954, on Matilda loam with a pH of 7.0. A fertilizer treatment of 4-24-12 at 250 pounds per acre was applied at the time of seeding and repeated again in September 1955. The plots were clipped three to five times per year to simulate grazing.

*Experiment 4, Ste. Anne de la Pocatière*

Eight mixtures, five of which are the same as in Experiment 3, were seeded in 1955, on Kamouraska clay. A 4-12-6 fertilizer was applied at the rate of 400 pounds per acre at seeding time and was not repeated thereafter. Each year the first crop was cut as early hay and the aftermath clipped once or twice to simulate grazing.

## EXPERIMENTAL RESULTS

Data are reported as total annual production only since the emphasis is placed on the over-all value of birdsfoot trefoil compared with other legumes in mixtures with various grasses. The distribution of yield among cuttings will be discussed in another paper.

*Experiment 1*

Data summarized in Table 1 show that alfalfa-timothy outyielded the birdsfoot trefoil mixtures in the first 2 years after seeding. However, in the third year, due to partial winterkilling of alfalfa and the survival of

TABLE 3. — PERCENTAGE GROUND COVERED (NOT INCLUDING BARE GROUND) BY BIRDSFOOT TREFOIL OR ALFALFA, IN NINE MIXTURES (ST. ANDRÉ GRAVELLY LOAM)

| Species and rate of seeding in lb. per acre | 1951 | 1952 | 1953 | 1954 | 1955 |
|---|---|---|---|---|---|
| Empire 4, Timothy 8 | 29 | 26 | 22 | 19 | 1 |
| Empire 4, Timothy 8, Red Clover 4 | 12 | 12 | 14 | 12 | 2 |
| Empire 4, Orchardgrass 10 | 28 | 32 | 35 | 27 | 2 |
| Empire 4, Meadow Fescue 10 | 12 | 24 | 25 | 21 | 2 |
| Empire 4, Red Fescue 10 | 38 | 38 | 38 | 23 | 6 |
| Empire 4, Ottawa 39 Fescue 10 | 53 | 49 | 48 | 40 | 7 |
| Empire 4, Kentucky Blue 5 | 53 | 56 | 50 | 40 | 8 |
| Empire 4, Bromegrass 15 | 43 | 47 | 47 | 35 | 9 |
| Alfalfa 6, Bromegrass 15 | 50 | 48 | 48 | 28 | 38 |

trefoil, the trefoil mixtures produced more herbage than the alfalfa-timothy mixture. This tendency was still obvious in the fourth and fifth year with the gradual disappearance of alfalfa. In the fifth year, birdsfoot trefoil plots looked vigorous even though several plants had disappeared through winterkilling.

The data in Table 1 also show that the addition of red clover to the timothy-trefoil mixture increased yields in the first year but brought about a yield decrease in the second year. In subsequent years both mixtures produced about equal yields.

*Experiment 2*

Yield data are summarized in Table 2 and the percentage ground covered by birdsfoot trefoil and alfalfa is given in Table 3 for each year. The results indicate that bromegrass-alfalfa by far outyielded the birdsfoot-trefoil mixtures in every year. The percentage ground covered shows that alfalfa persisted better than birdsfoot trefoil with 38 per cent cover in the fifth year as compared with 9 per cent for the best trefoil mixture. The relatively lower proportion of trefoil and of alfalfa in the sward during the fourth year (1954) reflects the presence of white clover which volunteered readily in that season.

Of the seven grasses used in simple mixtures with birdsfoot trefoil, red fescue produced the best average yield (3,308 pounds) followed closely by Ottawa 39 tall fescue (3,109), bromegrass (3,005), orchardgrass (2,968) and Kentucky bluegrass (2,918 pounds). Timothy and meadow fescue produced significantly lower yields than the other five.

The orchardgrass-trefoil mixture was one of the best in general appearance and produced excellent yields in spite of heavy losses through winterdamage, which decreased yields materially, especially at the first cutting.

*Experiment 3*

Results presented in Tables 4 and 5 show that there was no significant difference between the yield of trefoil-timothy, trefoil-brome and trefoil-Reed Canary, but the percentage contribution of trefoil was higher with timothy than with the other two grasses.

TABLE 4. — YIELDS IN POUNDS OF DRY MATTER PER ACRE OF NINE MIXTURES GROWN ON MATILDA LOAM, OTTAWA

| Mixture and rate of seeding in lb. per acre | 1955 | 1956 | 1957 | Av. |
|---|---|---|---|---|
| 1. Empire 5, Timothy 8 | 4548 | 6067 | 5301 | 5305 |
| 2. Empire 5, Reed Canary 8 | 3649 | 5430 | 5173 | 4751 |
| 3. Empire 5, Brome 12 | 4170 | 5653 | 5775 | 5200 |
| 4. Alfalfa 6, Brome 12 | 5033 | 6379 | 5882 | 5765 |
| 5. Timothy 8, Red Clover 4, Ladino 2 | 2930 | 4952 | 4378 | 4087 |
| 6. Timothy 8, Red Clover 4, Empire 5 | 3533 | 5570 | 5877 | 4994 |
| 7. Reed Canary 8, Alfalfa 2, Empire 5 | 5378 | 6217 | 5584 | 5726 |
| 8. Reed Canary 8, Alfalfa 2, Ladino 2 | 3407 | 5058 | 3971 | 4994 |
| 9. Timothy 6, Alfalfa 6, Red Clover 6, Ladino 1, Alsike 1 | 3340 | 5576 | 4803 | 4573 |
| L.S.D. (.05) | 572 | 295 | 376 | 581 |

Brome-alfalfa outyielded brome-trefoil in the first 2 years but there was no difference in the third year. The contribution data indicate that the additional yield of the brome-alfalfa mixture was due to the alfalfa portion, which contributed 82 per cent in the first year while trefoil contributed only 48 per cent. The trend of the legumes over the 3 years shows that alfalfa was a good initial competitor but dropped considerably each year while trefoil maintained its stand.

In Mixture 5 and 6 with red clover and timothy in common, it is apparent, from yield and contribution data, that trefoil performed better than Ladino. A comparison of production in Mixtures 1 and 6 shows that red clover suppressed the contribution of trefoil in the first 2 years.

A comparison of Mixtures 7 and 8, with alfalfa and Reed Canary in common, shows that the addition of trefoil resulted in a higher yield than the addition of Ladino. Ladino and alfalfa seemed to depress the contribution of each other in the first year. Complex Mixture 9 used as a check was less productive than the simple mixtures.

*Experiment 4*

In the series of mixtures reported in Table 6, the last four are standard hay-pasture mixtures recommended by the Quebec Seed Board. For the 3-year average, Mixture No. 5, containing alfalfa, gave the highest yield. Contribution data in Table 7 show that alfalfa could stand the competition of red clover in the first year and increase its contribution after the disappearance of red clover.

A comparison of Mixtures 7 and 8 with timothy and red clover in common shows that birdsfoot trefoil was superior to alsike especially in the third year.

A very similar observation can be made when comparing birdsfoot trefoil to ladino in Mixtures 6 and 7.

Comparison of Mixtures 1, 3 and 4 shows the superiority of timothy over Reed Canary grass and bromegrass, as a companion grass for birdsfoot trefoil.

TABLE 5.—PERCENTAGE CONTRIBUTION TO DRY MATTER YIELD OF EACH MIXTURE COMPONENT (MATILDA LOAM)

| Mixture No. | Year | Trefoil | Alfalfa | Red clover | Ladino | Alsike | Timothy | Brome | Reed Canary | Weeds |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1955 | 72 | — | — | — | — | 22 | — | — | 6 |
| | 1956 | 67 | — | — | — | — | 28 | — | — | 5 |
| | 1957 | 52 | — | — | — | — | 36 | — | — | 12 |
| 2 | 1955 | 49 | — | — | — | — | — | — | 44 | 7 |
| | 1956 | 50 | — | — | — | — | — | — | 45 | 5 |
| | 1957 | 48 | — | — | — | — | — | — | 45 | 7 |
| 3 | 1955 | 48 | — | — | — | — | — | 52 | — | — |
| | 1956 | 50 | — | — | — | — | — | 49 | — | 1 |
| | 1957 | 53 | — | — | — | — | — | 47 | — | — |
| 4 | 1955 | — | 82 | — | — | — | — | 18 | — | — |
| | 1956 | — | 69 | — | — | — | — | 31 | — | — |
| | 1957 | — | 61 | — | — | — | — | 37 | — | 2 |
| 5 | 1955 | — | — | 18 | 10 | — | 43 | — | — | 29 |
| | 1956 | — | — | 9 | 53 | — | 33 | — | — | 5 |
| | 1957 | — | — | 7 | 32 | — | 39 | — | — | 16 |
| 6 | 1955 | 24 | — | 32 | — | — | 27 | — | — | 17 |
| | 1956 | 57 | — | 10 | — | — | 26 | — | — | 7 |
| | 1957 | 50 | — | 15 | — | — | 26 | — | — | 8 |
| 7 | 1955 | 15 | 62 | — | — | — | — | — | 23 | — |
| | 1956 | 23 | 39 | — | — | — | — | — | 36 | 2 |
| | 1957 | 26 | 32 | — | — | — | — | — | 37 | 5 |
| 8 | 1955 | — | 38 | — | 6 | — | — | — | 53 | 3 |
| | 1956 | — | 17 | — | 36 | — | — | — | 46 | 1 |
| | 1957 | — | 15 | — | 22 | — | — | — | 55 | 8 |
| 9 | 1955 | — | 55 | 15 | 2 | 5 | 16 | — | — | 7 |
| | 1956 | — | 41 | 4 | 29 | 2 | 22 | — | — | 2 |
| | 1957 | — | 40 | 7 | 18 | 0 | 25 | — | — | 9 |

TABLE 6. — YIELDS IN POUNDS OF DRY MATTER PER ACRE OF EIGHT MIXTURES GROWN ON KAMOURASKA CLAY

| Mixture and seeding rate in lb. per acre | 1956 | 1957 | 1958 | Av. |
|---|---|---|---|---|
| 1. Empire 4, Timothy 8 | 3700 | 4320 | 4620 | 4214 |
| 2. Viking 4, Timothy 8 | 4520 | 3120 | 3600 | 3747 |
| 3. Empire 4, Reed Canary 8 | 3120 | 3300 | 3940 | 3453 |
| 4. Empire 4, Brome 12 | 3460 | 3500 | 4060 | 3673 |
| 5. Alfalfa 4.8, Red Clover 3.2, Timothy 8 | 5480 | 5380 | 6160 | 5673 |
| 6. Timothy 8, Red Clover 4, Ladino 2 | 4500 | 4260 | 3520 | 4093 |
| 7. Timothy 8, Red Clover 4, Empire Trefoil 4 | 4640 | 3960 | 4400 | 4333 |
| 8. Timothy 8.8, Red Clover 4.8, Alsike 2.4 | 4860 | 3760 | 2900 | 3840 |
| L.S.D. (.05) | 386 | 489 | 918 | 357 |

TABLE 7. — PERCENTAGE CONTRIBUTION TO DRY MATTER YIELD OF EACH MIXTURE COMPONENT (KAMOURASKA CLAY)

| Mixture No. | Year | Empire trefoil | Viking trefoil | Alfalfa | Red Clover | Ladino | Alsike | Timothy | Brome | Reed Canary | Others * |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 1956 | 28 | — | — | — | — | — | 37 | — | — | 35 |
| | 1957 | 40 | — | — | — | — | — | 60 | — | — | — |
| | 1958 | 34 | — | — | — | — | — | 62 | — | — | 4 |
| 2 | 1956 | — | 40 | — | — | — | — | 29 | — | — | 31 |
| | 1957 | — | 10 | — | — | — | — | 90 | — | — | — |
| | 1958 | — | 13 | — | — | — | — | 80 | — | — | 7 |
| 3 | 1956 | 25 | — | — | — | — | — | — | — | 32 | 43 |
| | 1957 | 40 | — | — | — | — | — | — | — | 60 | — |
| | 1958 | 28 | — | — | — | — | — | — | — | 68 | 4 |
| 4 | 1956 | 23 | — | — | — | — | — | — | 48 | — | 29 |
| | 1957 | 26 | — | — | — | — | — | — | 74 | — | — |
| | 1958 | 33 | — | — | — | — | — | — | 62 | — | 5 |
| 5 | 1956 | — | — | 38 | 47 | — | — | 14 | — | — | 1 |
| | 1957 | — | — | 46 | — | — | — | 54 | — | — | — |
| | 1958 | — | — | 50 | — | — | — | 45 | — | — | 5 |
| 6 | 1956 | — | — | — | 63 | 21 | — | 15 | — | — | 1 |
| | 1957 | — | — | — | 1 | — | — | 99 | — | — | — |
| | 1958 | — | — | — | — | 2 | — | 86 | — | — | — |
| 7 | 1956 | 1 | — | — | 74 | — | — | 23 | — | — | 2 |
| | 1957 | 7 | — | — | 1 | — | — | 92 | — | — | — |
| | 1958 | 32 | — | — | — | — | — | 62 | — | — | 6 |
| 8 | 1956 | — | — | — | 72 | — | 13 | 14 | — | — | 1 |
| | 1957 | — | — | — | 2 | — | — | 98 | — | — | — |
| | 1958 | — | — | — | — | — | — | 86 | — | — | 14 |

*Others: Mostly volunteer white clover which was killed during the winter 1956-57

The addition of red clover to trefoil-timothy significantly increased the yield in the first year (Mixture 1 vs. Mixture 7) but produced a lower yield in the second year and materially decreased the proportion of birdsfoot trefoil in the first and second year, (28 and 40 per cent respectively without red clover, and 1 and 7 per cent with red clover). However, in the third year, the contribution of birdsfoot trefoil increased to 32 per cent after red clover had disappeared.

Viking (Mixture No. 2) in the first year contributed more to dry yields than Empire (Mixture No. 1) — 40 and 28 per cent respectively — and produced significantly higher yields than Empire. However, in the second year, the Viking mixture produced significantly less as a result of extensive winterdamage; Viking contributed only 10 per cent to dry matter yields as compared to 40 per cent for Empire.

## DISCUSSION

The four experiments reported were conducted on three different soil types and at two locations, 350 miles apart. Due to this variety of conditions the behaviour of birdsfoot trefoil was not always consistent; yet trends were sufficiently similar to bring out some of its important characteristics.

It is evident that birdsfoot trefoil will not replace alfalfa on soils where the latter is adapted. Even on soils where drainage is imperfect, alfalfa mixtures outyielded birdsfoot trefoil mixtures in short term stands, as shown in Experiments 3 and 4.

Data obtained in three of these experiments show that the inclusion of red clover in a grass-birdsfoot trefoil mixture depresses the percentage contribution of trefoil to the crop. Results indicate that birdsfoot trefoil may recover from this detrimental competition and produce good yields in the third year but not in all cases. Red clover, therefore, should be used with great caution and its rate of seeding should be as low as possible to reduce competition with birdsfoot trefoil. In one experiment, where alfalfa instead of red clover was added to a simple mixture, the same competitive effect was observed on birdsfoot trefoil.

It was obvious that the establishment of birdsfoot trefoil was more satisfactory, as determined by per cent contribution to yield or per cent ground covered when it was associated with less aggressive grasses. Birdsfoot trefoil is slow growing in spring, and a quick growth of a companion species may offer too much competition. Timothy generally allowed the best establishment of trefoil and excellent yields were obtained from a timothy-birdsfoot trefoil mixture. There was an exception on St-André gravelly loam where timothy-birdsfoot trefoil gave poor results. This is difficult to explain, but it may be due to the sensitivity of timothy to drought, a condition prevailing on this soil type.

Good stands of birdsfoot trefoil were also obtained when it was associated with Kentucky blue grass, Ottawa 39 tall fescue, bromegrass, and red fescue, four slow-establishing grasses. However, these grasses had a tendency to crowd out trefoil after a few years. The productivity of bromegrass in association with trefoil varied according to the adaptation of this grass to the various soil types where it was grown.

Orchardgrass was grown in mixture with birdsfoot trefoil in only one test, on St-André gravelly loam. It performed surprisingly well, even though it partially winterkilled twice during the 5-year period, and it did not crowd out trefoil any more than other grasses. These results indicate the possible usefulness of this mixture in dry habitats, where orchard grass is sufficiently winter hardy. Because of its aggressiveness in the first year meadow fescue was one of poorer grasses to have in association with trefoil. Reed Canary grass in association with birdsfoot trefoil resulted in low yields at both locations and it depressed the contribution of trefoil more than timothy.

The value of birdsfoot trefoil lies in its persistence, which was better than that of alfalfa and red, alsike and ladino clovers in these experiments. There was one exception on St-André gravelly loam, where alfalfa persisted longer than trefoil. Actually, with most mixtures tried in all experiments, trefoil stands decreased gradually with years, showing that it will not persist indefinitely. It must be pointed out, however, that the uniform clipping height with the Gravely mower prevented the possible reseeding of trefoil, which often occurs under grazing conditions.

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# EARLY SEEDLING VIGOUR AND SEED WEIGHT IN RELATION TO BREEDING IN SMOOTH BROMEGRASS, *BROMUS INERMIS* LEYSS.[1]

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## ABSTRACT

Variability in early seedling vigour was studied in polycross progenies from highly selected plants rated as good in other agronomic characters and in open-pollinated progenies from previously unselected $S_0$ plants. Differences were found in both groups in rate and per cent emergence, and in height and vigour at 28 days. Considering the variability observed in these characters and the favourable associations found, it appears that early seedling vigour in bromegrass can be improved by selection within the species.

Seed weight was closely associated with early seedling vigour. Plants differed widely in seed weight, and in breeding for early seedling vigour it appears that most rapid progress can be made by screening breeding material for seed weight. Because seed weight did not account for all of the differences among progenies in seedling vigour, progenies from high seed weight plants or families should be subjected to a seedling vigour test to further select for this character. The close relationship found between greenhouse and field trials indicates that at least the initial screening can be done in the greenhouse.

## INTRODUCTION

The trend toward the use of simple mixtures in seeding hay and pasture crops places greater emphasis on the problem of stand assurance in each species. Although smooth bromegrass is relatively vigorous in the seedling stage compared with many other cool season grasses, it is sensitive to shade and increased seedling vigour would be a valuable asset. A more vigorous seedling could be expected to survive better under such normal hazards as deep seeding, puddling on heavy clay soils, early season moisture deficits and competition from weeds and associated crop plants.

Differences among strains of smooth bromegrass in early seedling vigour (2, 3, 11) and in later seedling vigour, that is 2 months or longer from seeding (7, 10), have been reported. Strains in other grass species also vary in early seedling vigour (5, 6, 12, 13) and in late seedling vigour (1, 4, 8, 14). Raeber (11) found that clones of smooth bromegrass differed in general combining ability for seedling vigour and, although this character was low in heritability, he concluded that selection for seedling vigour among and within strains would be effective.

Rogler (13) suggested that the best method of breeding for increased seedling vigour in crested wheatgrass was through selection of heavy-seeded types. He found a high positive correlation between seed weight and ability to emerge from deep plantings, the heavy-seeded strains being superior. Heavy seed-weight strains developed more rapidly when planted deep and produced sturdier seedlings at all depths than did the lighter seed-weight strains. In contrast, Lawrence (6) observed no relationship

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between seed weight and ability to emerge from deep seedings in intermediate wheatgrass lines. Kneebone (4) reported that selection for seedling vigour was effective in side-oats grama and that seed weight was correlated with seedling vigour. Seed weight was found to be a heritable character.

This study was initiated to investigate the extent of early seedling vigour variability in smooth bromegrass, to ascertain the feasibility of improving the seedling characters of this grass through breeding and to study the association of seed, seedling and maternal plant characters in relation to selection in a breeding program. Early seedling vigour in this study refers to vigour up to 4 weeks from seeding.

## MATERIALS AND METHODS

Three experiments, two in the greenhouse and one in the field, were conducted to study various aspects of seed weight and seedling relationships in bromegrass. All tests were of the randomized complete block design, three replications being used in the greenhouse trials and four in the field trials. Two depths of planting, 0.25 and 1.5 inches, were used to investigate a possible progeny x depth interaction.

The first experiment was designed to investigate variability in seedling vigour among progenies of plants selected as generally superior in type and disease reaction. Ten polycross seed lots were selected from a group of 50 produced in an isolated polycross nursery at Madison, Wisconsin, in which each clone had been entered 12-14 times. Selection of the 10 lots was made on the basis of variation in seed quality evident among the 50 seed lots after they had been threshed and cleaned uniformly. Seed weight determinations were made on three samples of 100 seeds each. Fifty seeds of each seed lot were planted in a medium-textured soil in the greenhouse in a 3-inch diameter clay pot at the two depths. Data were collected on rate of emergence, per cent emergence, height and vigour. Rate of emergence was calculated by expressing the number emerged at an early date as a percentage of the total emerging. The early date selected was when the variability, as measured by coefficient of variation, was reduced to a reasonable level. Seedling vigour was estimated using an index of 1 (low) to 10 (high). Seedling height was recorded in centimetres from the soil surface to the average height of the tallest 20 per cent of the seedlings in the pot.

The second experiment was designed to determine the extent of variability in early seedling vigour among seedling progenies from $S_0$ clones and to study seed, seedling and maternal plant character associations. Open-pollination (OP) seed was harvested from 100 $S_0$ plants of 572 in a single plant source nursery comprising 18 strains. The plants were selected to represent the range of variability present in this nursery in plant height, plant dry matter volume, leafiness, coarseness of stem, spread, seed yield per plant and fertility (percentage of fertile florets). From the group of 100 non-processed seed lots, 10 were selected with 100-seed weight values of 401-540 mg., 10 lots 370-379, 10 lots 320-329, and 10 lots 263-297, making four seed weight groups with mean weights per 100 seeds of 431

for Group I, 375 for Group II, 325 for Group III, and 281 mg. for Group IV. Eleven additional seed lots were included so that the total of fifty-one would provide progenies representative for all the plant and seed characters considered in selection of the original 100 plants. At least 5 plants representing high, and 5 representing low values in each character were included. The 51 OP seed lots were seeded in the greenhouse following the same procedure as described previously. Height, dry matter volume, leafiness, coarseness of stem, spread, seed yield per plant, fertility and seed weight data were taken on each of the 100 selected plants and these characters were correlated with the attributes of seed and seedling establishment. Percentage data were transformed prior to analysis, using the arcsin transformation.

To obtain further information on the extent to which seed weight determines seedling vigour, one $S_0$ plant was selected from each of the four seed weight groups for more detailed study. Three to four seed weight classes were established from the OP seed of each plant and these were seeded at an intermediate depth of .75 inches in the greenhouse.

A third experiment was conducted to relate the results of the greenhouse test to field performance. A field planting of 11 OP seed lots was made in each of 2 years to compare with a greenhouse trial. In the field trials an experimental unit consisted of 100 seeds planted in a 2-foot row with the rows spaced 6 inches apart. Uniform depth of seeding was accomplished with the aid of an excavator similar to that described by Moore (9).

TABLE 1. — SEED WEIGHT AND EARLY SEEDLING CHARACTERS OF TEN POLYCROSS PROGENIES GROWN IN THE GREENHOUSE

| Polycross progeny number | 100-seed weight, mg. | Rate of emergence from 0.25-inch depth[1] | Emergence[2], 28 days, transformed | Height, 28 days[2], cm. | Vigour at 28 days[3] | |
|---|---|---|---|---|---|---|
| | | | | | 0.25-inch depth | 1.5-inch depth |
| 31 | 385 | 45 | 69 | 21.9 | 10.0 | 7.6 |
| 17 | 361 | 47 | 66 | 19.2 | 10.0 | 6.3 |
| 3 | 333 | 67 | 73 | 20.3 | 10.0 | 5.6 |
| 12 | 305 | 57 | 68 | 20.2 | 10.0 | 5.6 |
| 25 | 302 | 54 | 65 | 18.6 | 10.0 | 3.6 |
| 41 | 281 | 52 | 62 | 18.0 | 9.0 | 3.6 |
| 42 | 280 | 67 | 73 | 19.7 | 9.7 | 5.6 |
| 20 | 274 | 32 | 53 | 19.1 | 9.3 | 5.6 |
| 28 | 265 | 60 | 67 | 20.2 | 10.0 | 7.0 |
| 15 | 243 | 41 | 56 | 16.4 | 8.0 | 2.0 |
| Mean | 303 | 52 | 65 | 19.4 | 9.6 | 5.3 |
| L.S.D. 0.05 | — | 15 | 6 | 1.3 | 0.9 | 1.5 |
| C.V. | — | 16.6 | 8.3 | 7.0 | 5.2 | 16.4 |

[1]Stand at 7 days as per cent of final stand, transformed values
[2]Mean over 0.25-inch and 1.5-inch depths of planting
[3]Vigour index 1 (*poor*) to 10 (*good*)

FIGURE 1. *Left to right*, polycross progenies 3 and 15 at the 0.25-inch depth, and progenies 3 and 15 at the 1.5-inch depth, respectively, at 14 days from seeding.

## EXPERIMENTAL RESULTS

### POLYCROSS PROGENIES

From the data in Table 1 it is evident that the polycross progenies varied in rate of emergence, per cent emergence, height, and vigour. Progenies reacted similarly at both depths in per cent emergence and in vigour and height at 14 days but a highly significant interaction was obtained in vigour at 28 days. This interaction involved differences in magnitude only (see Table 1) with the extremes represented by progenies 31 and 15 where the differences between 0.25- and 1.5-inch depths were 2.4 and 6.0, respectively. These progenies also were the extremes in seed weight, 31 being high and 15 being low.

Figure 1 illustrates the extent of the difference among these selected progenies in height and general vigour at 14 days from seeding. Progeny 3 was good, although not the best in rate of emergence and early vigour; progeny 15 was low in rate of emergence and low in other characters considered. The lack of uniformity in seedling height and the angle of emergence of the coleoptile at the 1.5-inch depth indicates that progeny 15 was markedly reduced in vigour at that seeding depth.

### OPEN-POLLINATION PROGENIES

*Variation among Progenies*

The analysis of variance data for the characters of seedling establishment studied in the 40 progenies is shown in Table 2. Differences were found among progenies for all characters. The two progenies compared in Figure 2 illustrate the extent of differences obtained in seedling vigour. Progeny 64 (seed weight group I) was the most vigorous progeny, and progeny 76 (seed weight group III) was one of the lowest, but not the lowest in seedling vigour.

TABLE 2. — ANALYSES OF VARIANCE (MEAN SQUARES) OF OP PROGENIES AT EACH OF TWO DEPTHS OF SEEDING IN THE GREENHOUSE

| Source of variation | df | % Emergence | | Rate of emergence | Height, 10 days | Height, 28 days | | Vigour, 28 days | |
|---|---|---|---|---|---|---|---|---|---|
| | | .25[1] | 1.5[1] | .25 | .25 | .25 | 1.5 | .25 | 1.5 |
| Progenies | 50 | 98.3[3] | 166.8[3] | 562.5[3] | 3.6[3] | 9.4[3] | 16.0[3] | 136.5[3] | 178.9[3] |
| *Among seed weight groups* | 3 | 135.4[3] | 466.5[3] | 949.9[3] | 30.8[3] | 100.4[3] | 118.3[3] | 865.0[3] | 1261.7[3] |
| Linear component | 1 | 148.0[2] | 1344.3[3] | 2773.5[3] | 91.1[3] | 245.1[3] | 338.7[3] | 2542.9[3] | 3719.6[3] |
| Quadratic component | 1 | 56.3 | 27.0 | 2.7 | 0.0 | 14.8[3] | 1.1 | 21.2 | 28.5 |
| Cubic component | 1 | 201.8[2] | 28.2 | 73.5 | 1.3 | 41.3[3] | 15.2[2] | 31.0 | 37.4 |
| *Among progenies in groups* | 36 | 99.5[3] | 145.8[3] | 590.5[3] | 2.1[3] | 3.3[3] | 9.3[3] | 99.2[3] | 102.7[3] |
| Group I | 9 | 45.8 | 158.8[3] | 548.2[3] | 3.3[3] | 2.5[3] | 5.8[2] | 269.9[3] | 74.5 |
| Group II | 9 | 66.7[2] | 93.9[2] | 652.6[3] | 2.9[3] | 6.2[3] | 14.1[3] | 63.8[3] | 113.4[2] |
| Group III | 9 | 175.3[3] | 135.0[3] | 767.8[3] | 1.1 | 1.1[2] | 8.4[3] | 49.9[3] | 141.4[3] |
| Group IV | 9 | 110.0[3] | 195.4[3] | 393.4[3] | 0.8 | 3.4[3] | 8.8[3] | 13.0 | 81.5 |
| Replications | 2 | 53.9 | 144.3 | 2160.1[3] | 6.2 | 10.0[3] | 9.7[2] | 73.2[3] | 40.0 |
| Error | 100 | 29.8 | 42.9 | 168.7 | 0.6 | 0.6 | 3.1 | 12.0 | 49.4 |
| C.V. | | 7.1 | 9.7 | 25.9 | 10.4 | 3.5 | 9.2 | 5.2 | 14.6 |

[1]Depth of seeding in inches
[2]P exceeds 0.05
[3]P exceeds 0.01

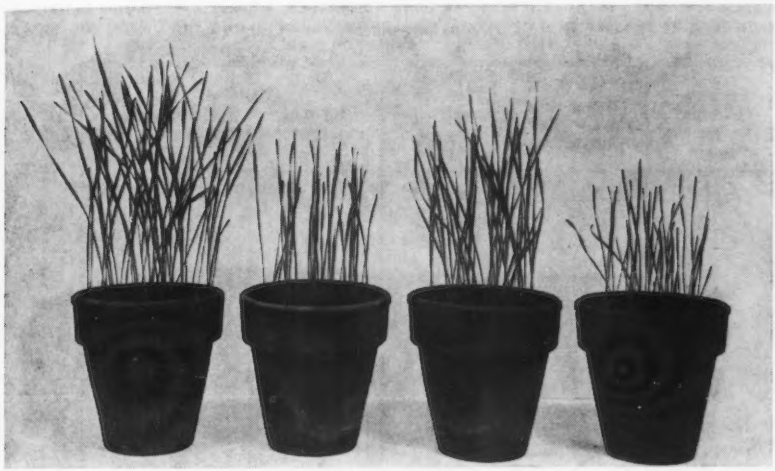

FIGURE 2. OP progenies 64 and 76, respectively, at the 0.25-inch depth on the *left*, and at 1.5 inches on the *right*, at 14 days from seeding.

Part of these differences among progenies could be attributed to seed weight. The relationship between seed weight and the characters of establishment was in most cases linear and the response curves are shown in Figure 3. In per cent emergence the cubic component of the variance among seed weight groups was significant for the 0.25-inch depth but not for the 1.5-inch depth of seeding. As evident in Figure 2 this was due to the fact that the heavy seed weight class did not have a higher percentage establishment than the medium-high class. At 10 days, increasing seed weight was associated with increasing seedling height in a linear manner. Although significant quadratic and cubic components were found in the height data at 28 days, the linear component was much larger, particularly at the 1.5-inch depth. Deviations from linearity were due to similar heights in the medium-high and high seed weight groups. At 32 days from seeding an overcrowding effect was clearly evident in these pots and it is possible that this effect occurred to a lesser degree prior to 28 days causing the most vigorous seedlings, the high seed weight group, to reduce growth relative to the other seedlings.

The effect of seed weight on seedling establishment attributes is further indicated in Table 3 where seed weight classes from the same seed lot are compared.

Seed weight did not account for all of the differences found among progenies. Differences occurred for all characters within most seed weight groups. Groups II and III provide the most reliable data on the variation among progenies of similar seed weight because of the narrow range of seed weight within each of these two groups. Significant differences were detected among progenies within each of these two groups for all characters, with one exception. No differences were found among progenies in Group III for height at 10 days.

TABLE 3. — RATE OF EMERGENCE, PER CENT EMERGENCE, HEIGHT AND VIGOUR RATINGS OF SEED WEIGHT CLASSES IN 4 OP PROGENIES AND CANADIAN BROME GROWN IN THE GREENHOUSE

| Progeny number or strain | Representing seed weight group number | 100-seed weight class, mg. | Rate of emergence[1] | % Emergence transformed | Vigour[2], 26 days |
|---|---|---|---|---|---|
| 64 | I | 617 | 83 | 80 | 10.0 |
| | | 480 | 72 | 82 | 9.0 |
| | | 394 | 79 | 76 | 8.7 |
| | | 297 | 72 | 65 | 7.0 |
| 99 | II | 447 | 87 | 85 | 9.3 |
| | | 360 | 81 | 82 | 7.2 |
| | | 228 | 75 | 71 | 4.5 |
| 76 | III | 410 | 62 | 74 | 6.7 |
| | | 315 | 45 | 62 | 3.5 |
| | | 203 | 35 | 40 | 1.0 |
| 14 | IV | 340 | 69 | 82 | 7.2 |
| | | 247 | 70 | 72 | 4.7 |
| | | 193 | 63 | 60 | 2.7 |
| Canadian Brome | — | 445 | 78 | 81 | 8.7 |
| | | 314 | 71 | 74 | 6.2 |
| | | 233 | 52 | 50 | 3.7 |
| L.S.D. 0.05 | | — | 15.8 | 7.8 | 0.7 |
| C.V. | | | 13.9 | 6.6 | 10.1 |

[1]Stand at 8 days from planting as per cent of final stand, transformed value
[2]Vigour index 1 (*poor*) to 10 (*good*)

TABLE 4. — VARIABILITY IN THE SEEDLING ESTABLISHMENT CHARACTERS AMONG 51 OP PROGENIES GROWN IN THE GREENHOUSE

| | Mean | Range | Standard deviation | Coefficient of variation |
|---|---|---|---|---|
| Rate of emergence | | | | |
| .25 inches[1] | 58 | 19-98 | 21.1 | 36.3 |
| 1.5 inches[2] | 68 | 10-94 | 19.4 | 28.5 |
| Per cent emergence | | | | |
| .25 inches | 95 | 77-99 | 4.9 | 5.2 |
| 1.5 inches | 85 | 62-99 | 9.2 | 10.9 |
| Height at 10 days (cm.) | | | | |
| .25 inches | 7.6 | 5.4-10.1 | 1.1 | 14.3 |
| 1.5 inches | 4.8 | 2.9-7.3 | 1.0 | 21.5 |
| Height at 28 days (cm.) | | | | |
| .25 inches | 22.4 | 18.8-26.6 | 1.8 | 7.9 |
| 1.5 inches | 19.0 | 14.9-23.8 | 2.6 | 13.5 |
| Vigour at 28 days[3] | | | | |
| .25 inches | 8.3 | 7.1-10.0 | 0.8 | 9.8 |
| 1.5 inches | 5.6 | 2.8-8.4 | 1.3 | 23.8 |

[1]Stand at 6 days as per cent of final stand
[2]Stand at 8 days as per cent of final stand
[3]Vigour index 1 (*poor*) to 10 (*good*).

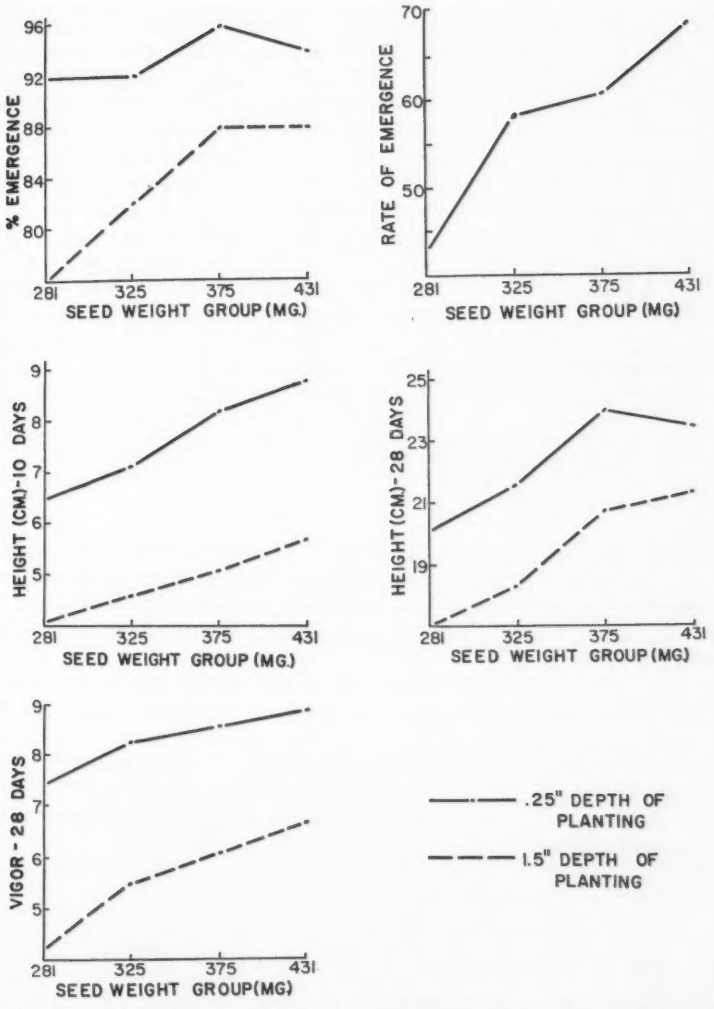


FIGURE 3. Relationships between seed weight and seedling establishment characters in 40 OP progenies grouped into four seed weight groups.

Similarly, from Table 3, differences in seedling vigour occurred which were not due to seed weight. For example, in progeny 64 the seed class of 297 mg. compared to progeny 76 seed weight class 315 mg. was superior in rate of emergence, height, and vigour. Comparison of the next highest seed weight class in these two progenies gave the same results. The latter comparison involves the highest seed weight class in progeny 76, a good level of seed weight, so that it did not include seed that was not completely

TABLE 5. — SIMPLE CORRELATION COEFICIENTS FOR RELATIONSHIPS AMONG MATERNAL PLANT AND OP PROGENY CHARACTERS AT THE 0.25-INCH DEPTH OF SEEDING

| Plant and progeny characters[2] | Rate of emergence[1] | % Emergence | Height, 10 days | Height, 28 days | Vigour, 28 days |
|---|---|---|---|---|---|
| Progeny characters | | | | | |
| Per cent emergence | +.25 | | +.36[4] | +.22 | +.33[4] |
| Rate of emergence[1] | | | +.75[4] | +.58[4] | +.49[4] |
| Vigour at 28 days | | | +.66[4] | +.59[4] | |
| Height at 28 days | | | +.98[4] | | |
| Maternal plant characters | | | | | |
| Dry weight | −.01 | +.18 | +.09 | +.19 | +.10 |
| Height | +.19 | +.11 | +.17 | +.23 | +.27[3] |
| Spread | +.06 | +.09 | +.07 | +.03 | −.15 |
| Leafiness | +.17 | +.09 | +.01 | −.01 | +.12 |
| Coarseness of culm | −.39[4] | +.14 | −.25 | +.30[3] | +.47[4] |
| Seed yield | +.51[4] | +.93[4] | +.31[3] | +.33[3] | +.37[4] |
| Fertility | +.40[4] | −.37[4] | +.19 | +.20 | −.20 |

[1]Stand at 6 days as per cent of final at .25 inches
[2]In seed yield n = 47 and in all other correlations n = 49
[3]P exceeds 0.05, i.e. .27 where n = 49
[4]P exceeds 0.01, i.e. .35 where n = 49

developed before harvesting. The extent of these differences in vigour is shown in Figure 4. Similar comparisons obtain in other progenies, such as between the intermediate seed classes of progenies 76 and 14. In this case, the latter, although lower in seed weight, was superior in all factors except per cent emergence.

A progeny x depth interaction was found in per cent emergence and in height and vigour at 28 days. In per cent emergence (see Figure 3) this interaction was due primarily to the superior ability of high seed weight material to establish at the deeper seeding. Progenies with the same seed weight reacted similarly at the two depths of seeding. However, in vigour and height at 28 days certain progenies were better able to withstand the 1.5-inch depth of seeding than were others. For example, at the two depths, the heights of progeny 64 were the same, while those of progeny 2 differed by 5 centimetres (24.6 per cent).

The variability among the 51 OP progenies in several seedling establishment characters is summarized in Table 4. The greatest variability, as measured by the coefficient of variation, was found successively in rate of emergence, height at 10 days, vigour at 28 days, height at 28 days, and per cent emergence. Variability was higher at the 1.5-inch depth of seeding than at the 0.25-inch depth in all characters except rate of emergence.

*Interrelation of Characters*

The character associations investigated and the simple correlation coefficients obtained are listed in Table 5. Several relationships were found among the progeny characters. Maximum stand was significantly correlated with height at 10 days and vigour at 28 days. Positive but not significant values were obtained with rate of emergence and height at 28

days. Rate of emergence was significantly related to height at 10 and 28 days and with vigour. Highly significant correlations were found for vigour with height at 10 and at 28 days. Height at the two intervals was closely related.

In the correlations of progeny with maternal plant characters no significant correlation coefficients were found with dry weight, height, spread, and leafiness. Coarseness of culm was correlated positively with height at 28 days and with vigour, and negatively with rate of emergence, indicating that coarse plants tended to produce progenies with low rates of emergence but good in height and vigour. However, because these correlation coefficients are low, the relationships are probably not useful in a selection program. Seed yield was correlated positively with rate of emergence, maximum emergence, height and vigour. Fertility was related positively with rate of emergence and negatively with maximum emergence indicating that plants classed as high in fertility tended to give progenies with high rates of emergence but low total emergence.

The correlation coefficients of seed weight with seven plant characters ($n = 100$) were as follows:

| | |
|---|---|
| Dry weight | $r = -.07$ |
| Height | $r = .24$ |
| Spread | $r = -.06$ |
| Leafiness | $r = .09$ |
| Number of culms | $r = -.21$ |
| Coarseness of culms | $r = -.11$ |
| Seed yield | $r = -.16$ |

Two values reached the 0.05 level of significance (.20) indicating a positive association with height and a negative one with number of culms. Both relationships were low.

FIGURE 4. Relative vigour of comparable seed weight classes within two OP progenies at 26 days from seeding. On the *left*, progeny 64 and 76 at 100-seed weights of 279 and 360 mgm., respectively; on the *right*, progeny 64 and 76 at seed weights of 394 and 410, respectively.

TABLE 6. — 100-SEED WEIGHT IN MG. OF OP SEED FROM THE $S_0$ PLANTS IN EACH OF THE FOUR SEED WEIGHT GROUPS AT DIFFERENT LOCATIONS AND IN DIFFERENT YEARS

| Seed weight group | Number of clones in group[1] | Location 1 | Location 2 | | | 4-year mean |
|---|---|---|---|---|---|---|
| | | 1951 | 1953 | 1954 | 1955 | |
| I | 8 | 433 | 499 | 473 | 378 | 446 |
| II | 9 | 374 | 451 | 408 | 357 | 398 |
| III | 8 | 325 | 396 | 411 | 346 | 370 |
| IV | 10 | 280 | 350 | 331 | 285 | 312 |

[1]10 in original group; only those retained for the 4-year period are shown here

TABLE 7. — SIMPLE CORRELATION COEFFICIENTS FOR THE RELATIONSHIP BETWEEN GREENHOUSE AND FIELD SEEDLINGS

| Seedling character correlated | Greenhouse 1952 with | |
|---|---|---|
| | Field 1951 | Field 1952 |
| Vigour | .80[2] | .83[2] |
| Height | .67[1] | — |
| Per cent emergence | | |
| 0.25-inch depth | .38 | .65[1] |
| 1.5-inch depth | .79[2] | .80[2] |

[1]P exceeds 0.05
[2]P exceeds 0.01

Heritability estimates for seed weight were not calculated in this material, but there was good consistency in ranking for seed weight of the plants in a non-replicated nursery in different years and locations as shown in Table 6. Inspection of the seed weight of individual plants in each year indicates that only a few were wrongly classified in the first year. A highly significant correlation ($r = .75$) was obtained between seed weight at the two locations (1951 and 1953) and between years with $r = .63$ for 1953 and 1954, .60 for 1953 and 1955, and .55 for 1954 and 1955. It is apparent that environmental differences did not markedly mask the expression of genotype for seed weight.

### SCREENING TECHNIQUE FOR EARLY SEEDLING VIGOUR

Preliminary trials on testing in rows in flats and in pots in greenhouse trials resulted in the selection of the 3-inch clay pot with 50 seeds as the experimental unit on the basis of compact grouping of seedlings for ease of obtaining vigour and height measurements, uniformity of seedling growth among replicates, accuracy of seeding at a specified depth and conservation of greenhouse space. A preliminary trial also indicated no seed lot x soil type interaction when non-sterilized sand, medium loam, and heavy clay soils were compared. A medium loam soil was used in subsequent trials.

Correlation coefficient of the results of greenhouse and field trials of 11 OP progenies are shown in Table 7. The good agreement shown by

these correlations indicates that screening for early seedling vigour can be done in the greenhouse with assurance that the data are indicative of field performance.

Seedling vigour in these trials was determined 4 weeks after planting. For study of seedling vigour at later stages it would be necessary to modify the experimental unit used in greenhouse trials because overcrowding effects on growth were evident soon after 4 weeks from seeding.

## DISCUSSION

The 10 polycross progenies tested came from a group of plants in an isolated polycross block. These plants had been previously selected as superior plants phenotypically, though not for seed characters. Differences in stand, height, and vigour were found in early seedling vigour among progenies of these plants. The differences were not large, but their existence indicates that seedling vigour differences can be found among plants rated as being generally superior.

The 51 OP progenies from $S_0$ plants differed in rate of emergence, per cent emergence, early height, and vigour at 28 days. In addition, in most cases these characters were closely associated so that it is possible to combine high rate of emergence, vigour, and per cent stand in one progeny. Considering the variability in these characters and the favourable association of characters found, it appears that early seedling vigour in bromegrass can be improved by selection within the species. The sample under study was not large and it is probable that an intensive search would provide breeding materials superior to the best obtained in this group.

A knowledge of the association of characters is often an aid to effective selection and some useful associations were found in this material. Seed weight was closely related to the attributes of establishment in the group of 40 OP progenies as evidenced by the highly significant among-seed-weight-group variances in all analyses. This relationship was found in all other trials as well. Eleven OP progenies grown in field trials provided correlation coefficients for seed weight and seedling vigour at 31 days of +.83 in 1951 and +.82 in 1952, both highly significant values. In the 10 polycross progenies a significant correlation ($r = +.60$) was found between seed weight and vigour at 28 days. The same relationship held among seed weight classes within seed lots.

Plant seed yield, fertility, height, and coarseness were correlated with some of the characters of establishment. However, most of the relationships were low and of little predictive value in a breeding program. Seed yield was the only one positively correlated with all attributes of seedling vigour but, even here, most of the correlation coefficients were not high. None of these maternal plant characters was as predictive of seedling vigour as was seed weight. Hence, in breeding for early seedling vigour, it appears that most rapid progress can be made by screening breeding material on the basis of seed weight.

Seed weight, however, did not account for all of the differences observed in seedling vigour. Progenies and seed classes of similar seed weight frequently differed in seedling vigour indicating that factors other than

seed weight were involved. In light of this, progenies from plants or families of similar seed weight should be grown to further select those with superior seedling vigour caused by factors other than seed weight. The close relationship found between greenhouse and field trials indicates that at least the initial screening can be done in the greenhouse. Both height measurements and vigour ratings provided, in general, satisfactory classification of progenies with respect of vigour. In screening for seedling vigour the rating has the advantage in that it includes an assessment of height, stem size, and leaf size. It was observed that certain progenies had taller seedlings having less stem diameter than other progenies. Hence, if height is used as an index of vigour, it would be desirable to check over the material for assurance that vigorous but shorter and stockier plants are not discarded.

It is clear that seed weight has an important effect on seedling establishment and vigour during the first 4-week period of development. This period is an extremely critical one in stand establishment. The duration of this effect of seed weight on seedling vigour has not been determined in this study and should be investigated. It is possible that factors other than seed weight play a greater role in determining seedling vigour at later stages than during this early stage.

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# THE GROWTH OF TOBACCO AT CERTAIN SOIL TEMPERATURES AND NUTRIENT LEVELS IN GREENHOUSE[1]

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**ABSTRACT**

Two varieties of tobacco, Hicks, a flue-cured variety, and Resistant Havana 211, a cigar variety, were grown at soil temperatures of 46°, 59°, 72° and 85°F. in a greenhouse. Three rates of each: N, $P_2O_5$, and $K_2O$ were applied in a factorial combination. The best soil temperature for growth was around 72°F. as indicated by measurements of leaf weight, leaf area, number of leaves, stalk weight, and stalk length. The 46°F. soil temperature was too low for good growth. Resistant Havana 211 grew better generally at the low and high soil temperatures. Phosphorus appeared to be the most important and beneficial nutrient element for growth at the lower soil temperatures.

## INTRODUCTION

Little information is available concerning the influence of soil temperature on growth of tobacco. Johnson and Hartman (3) investigated the influence of soil environment on the root-rot of tobacco. They found that in uninfested soil White Burley grew best at temperatures of 29° to 31°C., but poor growth was recorded at 13° and 40°C. With Connecticut Havana 28° to 29° was found to be the best soil temperature for top growth while the best root development took place at 23° to 24°C. In a similar study Jones *et al.* (4) also emphasized the influence of soil temperature on the incidence of root disease and subsequent reduction of yield. They noted that the optimum soil temperature for the growth of White Burley was between 20° and 25°C. Kincaid and Gratz (6) determined that, for the cigar varieties Round Tip and No. 301, the minimum growth temperature was 9°C. and the maximum about 40°C. while the optimum temperature was between 24° and 32°C.

The object of the work reported here was to evaluate the influence of soil temperature on growth of two tobacco varieties commonly grown in Canada, flue-cured variety Hicks and the cigar variety Resistant Havana 211(RH211), and to study what effects nitrogen, phosphorus and potassium may have on the growth of these varieties at different soil temperatures.

## MATERIALS AND METHODS

Cigar tobacco, variety Resistant Havana 211(RH211) and a flue-cured variety, Hicks, were seeded in quartz sand in the greenhouse September 3, 1958. After germination the seedlings were set out in soil in 3-inch porous clay pots. They were later transplanted into 1-gallon glazed clay pots, containing 4070 grams of air-dry sandy loam top soil. The soil was analysed with the following results:

| | |
|---|---|
| pH | 5.2 |
| N | 0.23 per cent |
| Organic matter | 5.52 per cent |
| Cation exchange capacity | 15.5 m.e. per 100 grams |
| Exchangeable K | 1.5 m.e. per 100 grams |
| $NaHCO_3$ soluble P | 47.8 p.p.m. |

[1]Contribution No. 26 from the Plant Research Institute, and Soils Research Institute, Research Branch, Canada Department of Agriculture, Ottawa, Ont.

The exchangeable K was considered to be very high and the soluble P was high.

The fertilizer treatment consisted of factorial combination of three rates of N, $P_2O_5$ and $K_2O$. The sources of nutrients were: N as $NH_4NO_3$ at rates of 0, 20, and 40 pounds per acre; $P_2O_5$ as $Ca(H_2PO_4)_2 . H_2O$ at rates of 0, 100 and 200 pounds per acre; $K_2O$ as $K_2SO_4$ at rates of 0, 120, and 240 pounds per acre. The fertilizer was mixed with the top 2 inches of soil.

The soil temperature was controlled in four baths at different temperatures: 46°F.(7.78°C.), 59°F.(15.0°C.), 72°F.(22.22°C.), and 85°F.(29.44°C.) (1). Each set of fertilizer treatments was used for both tobacco in each temperature bath, a total of 54 pots per bath. The pots were arranged in a confounded design with three blocks, as suggested by Yates (8).

The soil surface in the pots was insulated with beaded "Styrofoam"*. The day temperature of the air was generally maintained in the range of 75° to 80°F. and its night temperature at 70° to 75°F. Supplemental light of about 400-foot candles at the plant tips was provided to maintain a 12-hour day. The pots were watered and weighed regularly to maintain the soil moisture close to the moisture equivalent. The temperature treatments were begun October 21. The plants were harvested at about the flower-bud stage. Harvesting started November 25 and was completed December 3. Plant height, number of leaves, and total leaf area per plant at harvest were measured. Leaf area was estimated by tracing the leaves on paper and cutting and weighing the paper. The tissues were oven-dried and weights of leaves and stalks recorded. Roots were examined for disease infection but their weights were not determined.

*Supplied by D. Fraser & Co., Ottawa, Ont.

TABLE 1. — TOBACCO LEAF WEIGHT AS INFLUENCED BY SOIL TEMPERATURE AND NUTRIENT TREATMENTS

| Nutrient treatments | Temperature, degrees F., and average leaf weight, in grams | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 85 | | 72 | | 59 | | 46 | |
| | RH211 | Hicks | RH211 | Hicks | RH211 | Hicks | RH211 | Hicks |
| $N_0$ + PK | 8.11 | 8.89 | 12.46 | 8.87 | 7.99 | 6.42 | 0.65 | 0.82 |
| $N_{20}$ + PK | 7.72 | 8.48 | 12.24 | 9.14 | 9.23 | 6.48 | 0.61 | 0.90 |
| $N_{40}$ + PK | 7.68 | 8.91 | 12.17 | 9.03 | 8.03 | 5.47 | 0.56 | 0.77 |
| $P_0$ + NK | 8.02 | 7.63 | 11.51 | 8.14 | 6.99 | 4.71 | 0.58 | 0.53 |
| $P_{100}$ + NK | 7.81 | 8.98 | 12.00 | 9.60 | 9.07 | 6.29 | 0.61 | 1.01 |
| $P_{200}$ + NK | 7.67 | 9.66 | 13.36 | 9.30 | 9.19 | 7.37 | 0.63 | 0.96 |
| $K_0$ + NP | 7.86 | 8.42 | 12.48 | 9.69 | 8.77 | 5.57 | 0.61 | 0.72 |
| $K_{120}$ + NP | 6.76 | 9.18 | 12.05 | 9.09 | 8.18 | 6.50 | 0.54 | 0.98 |
| $K_{240}$ + NP | 8.01 | 8.68 | 12.34 | 8.26 | 8.30 | 6.31 | 0.66 | 0.78 |
| Significant effects: | Variety | | Variety* | | Variety* | | Variety | |
| 5% level | | | P | | N | | P | |
| 1% level* | | | NP* | | P* | | | |
| | | | | | NK | | | |
| | | | | | PK | | | |

As there was only one replication of temperature baths the growth measurements can be compared statistically only for pots in each bath and not between baths. The effects of temperature on growth were generally quite large, however, so comparative estimates can be made. The data have been analysed by methods suggested by Fisher (2) and Yates (8).

## RESULTS AND DISCUSSION

Growth measurements of the tobacco plants are recorded in Tables 1 to 5. Those of economic importance, i.e., leaf weight, leaf area, and number of leaves are discussed in the following paragraphs.

Visual examination of the plants during the course of the experiment showed that the 72°F. soil temperature was the best for growth. A decrease was evident in most of the growth measurements at the 85°F. and 59°F. soil temperatures. A drastic reduction of growth occurred at the 46°F. soil temperature.

### *Leaf Weight*

Hicks outyielded RH211 at 85° and 46°F. but the reverse was true at 72° and 59°F. soil temperature. Leaf weight was increased, although non-significantly by P applications at the 85°F. temperature. At this temperature, with increasing amounts of P, Hicks produced more weight of leaves but there was no change with RH211. At the 72°F. temperature the leaf weight was significantly increased by P application. NP interactions were highly significant at this temperature: N with $P_0$ and $P_{200}$ treatments decreased, but with $P_{100}$ increased the leaf weight. The greatest leaf weights at this temperature were obtained with $P_{200}$ and $N_0$, and with $N_{40}$ and $P_{100}$ applications; $N_{40}$ without P gave the lowest yield. At the 59°F. temperature N decreased the leaf weight while P increased it to a

TABLE 2. — TOBACCO LEAF AREA AS INFLUENCED BY SOIL TEMPERATURE AND NUTRIENT TREATMENTS

| Nutrient treatments | Temperature, degrees F., and average leaf area dcm² | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 85 | | 72 | | 59 | | 46 | |
| | RH211 | Hicks | RH211 | Hicks | RH211 | Hicks | RH211 | Hicks |
| $N_0$ + PK | 32.48 | 31.00 | 50.79 | 35.22 | 34.52 | 22.39 | 1.93 | 1.94 |
| $N_{20}$ + PK | 31.11 | 30.73 | 54.59 | 36.23 | 37.67 | 25.81 | 1.87 | 2.06 |
| $N_{40}$ + PK | 31.50 | 31.81 | 54.83 | 35.25 | 33.41 | 21.97 | 1.82 | 1.73 |
| $P_0$ + NK | 31.70 | 26.79 | 50.21 | 31.36 | 29.56 | 20.00 | 1.83 | 1.20 |
| $P_{100}$ + NK | 30.25 | 32.97 | 54.20 | 37.90 | 36.66 | 24.33 | 1.80 | 2.53 |
| $P_{200}$ + NK | 33.15 | 33.80 | 55.80 | 37.44 | 36.64 | 27.58 | 1.99 | 2.00 |
| $K_0$ + NP | 30.55 | 30.12 | 54.17 | 38.16 | 34.24 | 22.39 | 1.82 | 1.00 |
| $K_{120}$ + NP | 31.66 | 32.59 | 55.56 | 34.36 | 34.96 | 23.89 | 1.78 | 2.25 |
| $K_{240}$ + NP | 32.88 | 30.85 | 50.49 | 34.18 | 34.66 | 25.63 | 2.02 | 1.88 |
| Significant effects: | | | Variety* | | Variety* | | | |
| 5% level | | | P | | N* | | | |
| 1% level* | | | NP | | P* | | | |
| | | | | | NK* | | | |

highly significant extent. NK interactions were significant at this temperature: with $K_{120}$ the N treatments decreased the leaf weight, with $K_0$ and $K_{240}$ treatments $N_{20}$ was beneficient but $N_{40}$ detrimental; where there was no N the $K_{120}$ treatment was beneficial and $K_{240}$ detrimental; the harmful influence of $N_{40}$ treatments was suppressed by K. At the 46°F. temperature P treatments increased the weight of leaves significantly.

*Leaf Area*

Leaf area was not significantly influenced by fertilizer treatments at the 85° and 46°F. At 72°F., P produced an increase in leaf area. Highly significant NP interactions showed that $P_{100}$ and $P_{200}$ treatments overcame the deleterious influence of $N_{20}$ and $N_{40}$ treatments; the highest values were obtained with $N_{20}$ and $N_{40}$ and $P_{100}$; but the lowest values with $N_{40}$ and without P. At the 59°F. temperature N applications produced very highly significant differences in leaf area: $N_{20}$ gave an increase and $N_{40}$ a decrease below that of $N_0$ value. P caused a highly significant increase in leaf area. NK interactions showed that where $N_{40}$ decreased the leaf area $K_{240}$ neutralized this effect; K applications increased the leaf area where no N was applied. RH211 produced highly significantly greater leaf area than Hicks at the 72° and 59°F. temperatures; at 85° and 46°F. this was reversed but the differences were non-significant.

*Number of Leaves*

RH211 produced a greater number of leaves at 85°, 72°, and 59°F., than at 46°F. but the differences were significant only at the 72° and 59°F. temperatures. At the 46°F. temperature Hicks had significantly more leaves than RH211. At the 59°F. temperature P increased the number of leaves to a significant degree. NK interactions were significant at this temperature: K treatments in presence of N generally increased the number

TABLE 3.—NUMBER OF LEAVES OF TOBACCO PLANTS AS INFLUENCED BY SOIL TEMPERATURE AND NUTRIENT TREATMENTS

| Nutrient treatments | Temperature, degrees F., and average number of leaves | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 85 | | 72 | | 59 | | 46 | |
| | RH211 | Hicks | RH211 | Hicks | RH211 | Hicks | RH211 | Hicks |
| $N_0$ + PK | 15.22 | 14.77 | 17.77 | 14.77 | 14.22 | 12.55 | 7.11 | 8.00 |
| $N_{20}$ + PK | 15.55 | 15.00 | 17.44 | 14.88 | 16.66 | 12.33 | 7.22 | 8.22 |
| $N_{40}$ + PK | 15.88 | 14.88 | 17.77 | 14.33 | 14.77 | 12.00 | 6.66 | 8.11 |
| $P_0$ + NK | 15.77 | 14.33 | 17.11 | 14.88 | 14.22 | 12.00 | 6.55 | 7.77 |
| $P_{100}$ + NK | 15.44 | 15.22 | 18.11 | 14.33 | 15.11 | 12.66 | 7.22 | 8.55 |
| $P_{200}$ + NK | 15.44 | 15.11 | 17.77 | 14.77 | 15.66 | 12.80 | 7.22 | 8.00 |
| $K_0$ + NP | 15.11 | 14.55 | 17.66 | 15.11 | 14.88 | 12.22 | 7.11 | 7.66 |
| $K_{120}$ + NP | 15.33 | 15.11 | 17.77 | 14.44 | 15.11 | 12.44 | 7.00 | 8.55 |
| $K_{240}$ + NP | 16.22 | 15.00 | 17.55 | 14.44 | 15.00 | 12.88 | 6.88 | 8.11 |
| Significant effects: | | | | Variety* | | Variety* | | Variety* |
| 5% level | | | | | | P | | |
| 1% level* | | | | | | NK | | |

TABLE 4.—TOBACCO STALK WEIGHT AS INFLUENCED BY SOIL TEMPERATURE AND NUTRIENT TREATMENTS

| Nutrient treatments | Temperature, degrees F., and average stalk weight, in grams | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 85 | | 72 | | 59 | | 46 | |
| | RH211 | Hicks | RH211 | Hicks | RH211 | Hicks | RH211 | Hicks |
| $N_0$ + PK | 5.28 | 4.74 | 6.28 | 4.51 | 3.72 | 2.65 | 0.19 | 0.31 |
| $N_{20}$ + PK | 4.81 | 4.89 | 6.68 | 4.68 | 3.92 | 2.71 | 0.22 | 0.35 |
| $N_{40}$ + PK | 5.07 | 4.78 | 6.38 | 4.26 | 3.50 | 2.42 | 0.19 | 0.29 |
| $P_0$ + NK | 5.07 | 4.46 | 5.79 | 4.48 | 3.03 | 1.99 | 0.18 | 0.23 |
| $P_{100}$ + NK | 5.18 | 4.61 | 6.42 | 4.61 | 4.11 | 2.66 | 0.19 | 0.41 |
| $P_{200}$ + NK | 4.91 | 5.34 | 7.13 | 4.37 | 4.00 | 3.13 | 0.23 | 0.31 |
| $K_0$ + NP | 5.34 | 4.81 | 6.64 | 5.02 | 4.04 | 2.23 | 0.20 | 0.30 |
| $K_{120}$ + NP | 5.00 | 4.74 | 6.47 | 4.32 | 3.63 | 2.77 | 0.19 | 0.37 |
| $K_{240}$ + NP | 4.82 | 4.86 | 6.23 | 4.13 | 3.47 | 2.78 | 0.21 | 0.28 |
| Significant effects: | | | | Variety* | Variety* | | Variety | |
| 5% level | | | | | P* | | P | |
| 1% level* | | | | | Variety x K | | NP | |
| | | | | | | | Variety x N | |
| | | | | | | | Variety x P* | |
| | | | | | | | Variety x K* | |

of leaves although the largest number of leaves at this temperature was obtained with $N_{20}$ and $K_0$.

*General*

Fertilizer treatments often produced different growth responses at each of the four soil temperatures tested. At some temperatures an application of a nutrient element and treatment would cause an increase in growth while at other temperatures the same treatment would cause a decrease. Generally where the growth was hindered by high or low root temperatures the plants responded somewhat similarly to nutrient treatment. When responses of plants were compared between those grown at a growth-hindering and those grown at some intermediate temperature, i.e., 85° vs. 59°F., 72° vs. 46°F. or 59° vs. 46°F., they often were quite dissimilar.

With all growth measurements recorded the best soil temperature for top growth was around 72°F.(22-23°C.). This temperature agrees well with optimum temperatures for growth of White Burley (3) but is lower than the optimum temperature reported for Connecticut Havana, Round Tip, or No. 301 (6). The temperature of 46°F. or approximately 8°C. was too low for good growth, although the variety Hicks reached the same physiological stage of growth here as in the higher temperatures. Growth at the low temperatures may have been caused by the poor growth of roots and the retarded ability to take up water which was indicated by wilting of plants at the start of this experiment.

TABLE 5.—HEIGHT OF TOBACCO PLANTS AS INFLUENCED BY SOIL TEMPERATURE AND NUTRIENT TREATMENTS

| Nutrient treatments | Temperature, degree F., and average plant height in cm. | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 85 | | 72 | | 59 | | 46 | |
| | RH211 | Hicks | RH211 | Hicks | RH211 | Hicks | RH211 | Hicks |
| $N_0$ + PK | 67.88 | 64.88 | 73.61 | 70.72 | 55.27 | 48.88 | 5.00 | 14.55 |
| $N_{20}$ + PK | 65.55 | 67.11 | 81.66 | 70.55 | 52.22 | 50.16 | 6.00 | 15.24 |
| $N_{40}$ + PK | 62.88 | 65.72 | 77.00 | 67.11 | 54.11 | 49.55 | 4.66 | 12.94 |
| $P_0$ + NK | 61.66 | 62.77 | 73.33 | 71.05 | 52.61 | 47.83 | 4.77 | 11.00 |
| $P_{100}$ + NK | 67.22 | 66.00 | 76.33 | 69.55 | 54.66 | 49.88 | 4.33 | 17.16 |
| $P_{200}$ + NK | 67.44 | 68.94 | 82.61 | 67.77 | 54.33 | 50.88 | 6.55 | 14.50 |
| $K_0$ + NP | 67.88 | 63.83 | 78.27 | 71.16 | 56.00 | 45.38 | 4.44 | 13.83 |
| $K_{120}$ + NP | 65.94 | 68.77 | 79.22 | 69.44 | 54.27 | 50.27 | 5.72 | 15.83 |
| $K_{240}$ + NP | 62.50 | 65.11 | 74.77 | 67.77 | 51.33 | 52.94 | 5.50 | 13.11 |
| Significant effects: | | | Variety | | Variety | | Variety* | |
| 5% level | | | NPK | | | | P | |
| 1% level* | | | | | | | NP | |
| | | | | | | | Variety x N | |
| | | | | | | | Variety x P | |

The RH211 variety grew better than Hicks at the intermediate temperatures while Hicks performed better at the low and high soil temperature. The exceptions were stalk weight and number of leaves where, even at the highest temperature, RH211 yielded more than Hicks.

The 85°F. temperature minimized the effects of fertilizer treatments as there were no significant effects. At the lower root temperatures, the supply of phosphorus appeared to be a critical nutrient element even in this rather rich soil. This is in good agreement with Ketcheson (5) and Shtrausberg (7) who found, working with corn, oats, rye, cabbage, and tomatoes, that with relatively low root temperature phosphorus, but not nitrogen and potassium, uptake may be hindered. Part of these effects might have been caused by P increasing the growth of roots, and part ascribed to the essential role of P in energy relationships in the plant. Nitrogen applications were important only at the 59°F. temperature. There is a probability that the better growth of plants at the 72°F. and 85°F. temperatures, than at the 59°F. temperature, reduced the detrimental effect of nitrogen to an insignificant level. At 46°F. the plant growth was retarded more than at 59°F. but the significant detrimental influence of nitrogen may have been masked by the generally poor growth. Potassium did not exert any influence. This was probably due to the high original content of K in the soil.

With tobacco culture expanding northwards, the knowledge of optimum and minimum soil temperature for growth of tobacco of different

varieties is important. Average soil temperature at 4-inch depth under sod at Ottawa, Ontario, L'Assomption, Quebec, and Harrow, Ontario were*:

| | Ottawa | L'Assomption, Que. | Harrow, Ont. |
|---|---|---|---|
| June 1 | 62.5 | 63.5 | 64.8 |
| June 15 | 68.9 | 69.4 | 73.2 |
| July 1 | 69.5 | 70.0 | 77.2 |
| July 15 | 71.4 | 73.5 | 75.9 |
| Aug. 1 | 72.8 | 73.8 | 75.8 |
| Aug. 15 | 69.7 | 71.9 | 75.4 |

Considering the data of this experiment, the soil temperatures at Ottawa and L'Assomption barely approach or are lower than the optimum temperatures for the varieties tested and are too low for the varieties Connecticut Havana, Round Tip, and No. 301. Under these conditions it is probable that liberal use of phosphorus fertilizers may improve the growth and yield of tobacco.

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# RELATIVE DECLINE OF *OPHIOBOLUS GRAMINIS*, *HELMINTHOSPORIUM SATIVUM* AND *FUSARIUM CULMORUM* IN THE SOIL[1]

G. SEMENIUK[2] AND A. W. HENRY[3]

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## ABSTRACT

The relative decline of three cereal root pathogens, *Ophiobolus graminis* Sacc., *Helminthosporium sativum* P. K. B., and *Fusarium culmorum* (W. G. Sm.) Sacc., in natural and sterilized black loam soil, was studied using inoculum grown in a sterilized soil-cornmeal medium. Since the severity of infection of wheat seedlings by the above fungi proved directly proportional to the amount of inoculum added to natural soil, it was used as a measure of the amount of effective inoculum in the soil at a given time. It was found by this means that inoculum of all three pathogens diminished greatly in amount in natural soil during the first few days following its addition, with that of *O. graminis* declining least, that of *H. sativum* more, and that of *F. culmorum* most. The decline rate was proportional to the amount of inoculum. After one week the decline had progressed further, but it was less for *O. graminis* than for the other two pathogens. In 3-4 weeks the amount of all three had reached a near-zero quantity. In sterilized soil the decline was similar to that in natural soil after the sterilized soil became recontaminated. However, that of *F. culmorum* was less pronounced here than in natural soil. Marked decline of *O. graminis* also occurred in natural soil supporting a seedling wheat crop. In large measure the decline studied appeared to be a quantitative degenerating process resulting from the activity of soil micro-organisms.


## INTRODUCTION

*Ophiobolus graminis* Sacc., *Helminthosporium sativum* P. K. & B., and *Fusarium culmorum* (W. G. Sm.) Sacc. are three pathogenic fungi that invade the roots and basal parts of the stems of wheat and other grass plants and reduce both yield and quality of affected hosts. Though it has been held in the past that such fungi tend to grow in the soil with continuous cropping to the same or related crops, a more recent view is that they grow mainly as they colonize plant parts and decline as these parts become invaded and disintegrated by the micro-organisms of the soil.

Various workers (1, 2, 3, 4, 10, 11, 15, 16, 17, 18) have found that artificially prepared inoculum of *O. graminis, H. sativum* or *F. culmorum* was ineffective after a time in natural or contaminated sterilized soil. This has been mainly attributed by the authors cited and by Garrett (7, 8, 9) to the antagonistic activity of other soil microbes. Different modes of action of the antagonists have been suggested. Garrett (5) ascribed the loss to actual bacterial decomposition of the pathogenic fungi or to their inactivation by toxic substances excreted by the other micro-organisms. He (6) later postulated for *O. graminis* that if this fungus is present in the soil and is not growing then it must be decomposing. He (5) also surmised that different foot- and root-rot fungi may vary considerably in their susceptibility to biological antagonisms.

In the present experiments the relative decline rate of inocula of the above three pathogens was followed in both natural and sterilized soil.

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[2]Professor of Plant Pathology, South Dakota State College of Agriculture and Mechanical Arts, Brookings, S. Dakota; formerly Graduate Student at the University of Alberta.

[3]Professor of Plant Pathology, University of Alberta, Edmonton, Alta.

## MATERIALS AND METHODS

Single virulent isolates of the three pathogens were grown for three weeks on a sterile, 5 per cent cornmeal-soil mixture and at the end of that time 2, 10, 30, 60 and/or 100 grams of the overgrown mixture were added as inoculum to potted natural and sterilized Edmonton black loam soil. Details of the procedure were as follows: Edmonton soil was mixed with washed riverbed sand in a 3:1 proportion; pots were 6-inch and of unglazed clay; potted soils were steam-sterilized for 8 hours at 15 pounds pressure; inocula were evenly layered 1 inch below the soil surface; inoculated and uninoculated soils were watered from above and held moist. All experiments were conducted in a tightly-fitted artificially-lighted room held at 17° to 20°C.

As will be seen below, the severity of wheat seedling infection proved directly proportional to the amount of inoculum. Accordingly, assessment of inoculum decline in the soil was judged from the severity of such infections at the start and at selected weekly intervals thereafter. Twenty-five Marquis wheat seeds, 1 year old and treated with 1:320 formaldehyde,

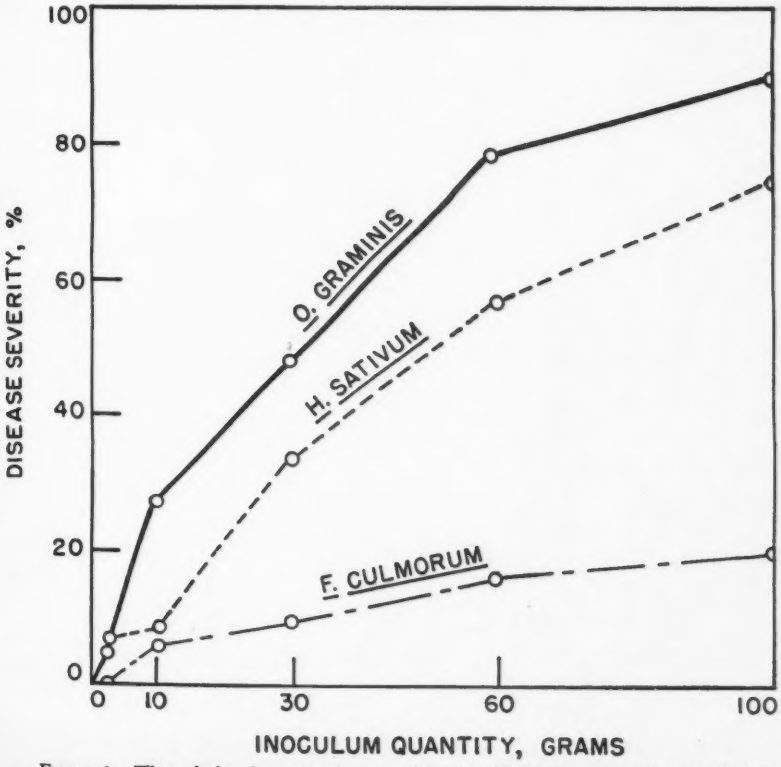


FIGURE 1. The relation between the quantity of artificial inoculum of each of three pathogens in natural soil and severity of infection of wheat seedlings.

were pushed with wooden sticks to the region of the inoculum in each of six randomly selected pots for each amount of inoculum. Three weeks later the severity of seminal root necrosis of individual seedlings was rated and summarized by the method of McKinney and Davis (13).

## EXPERIMENTAL RESULTS

Six different tests were conducted to compare the decline of inoculum of each of the three pathogens in natural and in sterilized soil. The results were similar for all, so only those of more complete tests are presented. Infections of check plants with no inoculum added are not presented but they were zero or very nearly zero for all.

*Disease severity as a measure of inoculum quantity*

Seeds were sown in natural soil immediately after the inoculum was added to it. As shown in Figure 1 for each of the pathogens, disease

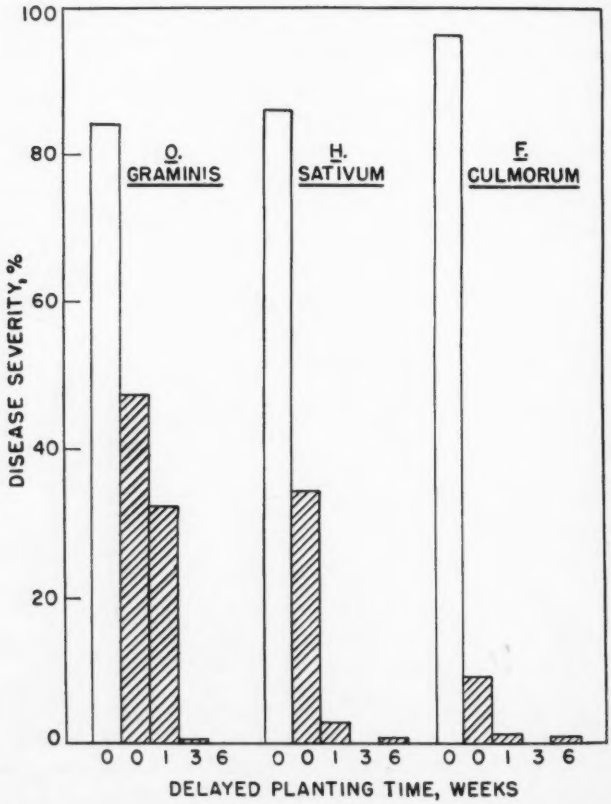


FIGURE 2. The comparative decline of 30 grams inoculum of each of three pathogens in natural soil (*shaded bars*) as may be judged from the infection of wheat seedlings by comparable inoculum in uncontaminated sterilized soil (*open bars*).

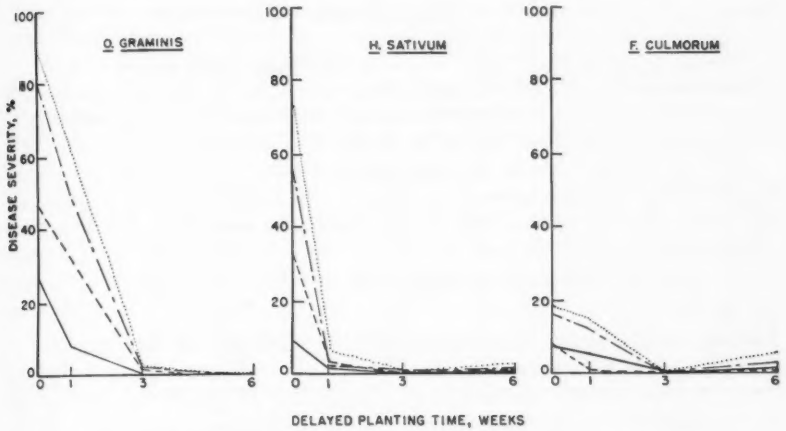


FIGURE 3. The comparative decline of 10 (—), 30 (---), 60 (- - - -) and 1000 (. . . .) grams inoculum of each of three pathogens added to natural soil.

severities increased curvilinearly with increases in inoculum quantity. Accordingly, disease severity may be taken to reflect inoculum quantity and, conversely, inoculum quantity may be taken to determine disease severity. On this basis a timely decline of disease severities in the following experiments was interpreted to signify a corresponding decline of inoculum amount.

The inocula of the three pathogens in sterilized soil appeared nearly equal at the start. In the natural soil, the quantities at the start proved unequal and lower than in sterilized soil. This difference in amount probably was the result of the action of soil micro-organisms on the inoculum in natural soil in the interval between seeding and infection of young seedlings. This interval may have been as much as 5 days corresponding to the time from sowing to shortly after plumule emergence.

The order of inoculum decline in natural soil at the start was least for *O. graminis*, greater for *H. sativum*, and greatest for *F. culmorum*. This is the same order as for the position of the curves for the pathogens in Figure 1, which is further evidence that the inocula declined in that order.

After 1 week the inoculum declined in sterilized soil and it declined further in natural soil. The decline in sterilized soil was believed to be the result of soil recontamination by micro-organisms from the air and tap water (2, 11). *O. graminis* did not decline appreciably in sterilized soil in this time while *H. sativum* and *F. culmorum* declined to near zero.

After 3 weeks, *O. graminis* reached near zero quantity in both natural and sterilized soil; *H. sativum* reached this low level earlier in both soils; while *F. culmorum* reached this low level earlier in natural soil, but it failed to decline to this extent in sterilized soil even after 6 weeks. The failure

TABLE 1. — INOCULUM DECLINE OF THREE PATHOGENS IN STERILIZED AND NATURAL SOIL

| Time of seeding after inoculum was added to soil | Disease severity% | | |
|---|---|---|---|
| | *O. graminis* | *H. sativum* | *F. culmorum* |
| | *Sterilized soil* | | |
| Immediately | 84.2 | 86.4 | 96.0 |
| 1 week | 83.9 | 14.0 | 21.9 |
| 3 weeks | 0.8 | 0.8 | 10.8 |
| 6 weeks | 0.0 | 1.6 | 22.0 |
| | *Natural soil* | | |
| Immediately | 47.4 | 34.6 | 8.8 |
| 1 week | 32.0 | 2.8 | 1.2 |
| 3 weeks | 0.5 | 0.0 | 0.0 |
| 6 weeks | 0.0 | 0.5 | 0.6 |

TABLE 2. — DECLINE OF *O. GRAMINIS* INOCULUM IN NATURAL SOIL SUPPORTING A SEEDLING WHEAT CROP

| Time of seeding first seedling crop | Disease severity, % | |
|---|---|---|
| | First seedling crop | Second seedling crop |
| Immediately | 80.7 | 28.8 |
| 2 weeks | 67.4 | 0.0 |
| 4 weeks | 15.4 | 3.7 |

of *F. culmorum* to decline completely in sterilized soil possibly was the result of the saprophytic growth it made in this soil.

*Rate of inoculum decline in natural soil*

Ten, thirty, sixty, and one hundred grams of inoculum of each of the three pathogens were added to natural soil. These amounts in sterilized soil at the start produced near maximum disease severities, which meant that three high amounts at least were superabundant for production of such severities. The results from natural soil, shown in Figure 3, therefore may be interpreted to show that the inoculum declined at a rate that was approximately proportional to the amount present. The rate was not constant over the entire period, however, but was higher at the beginning than later. Perhaps the change in rate at these times may have been due either to chlamydospore-type cells in the inoculum that resisted decomposition more than did the mycelium or to an ecologically-induced change in the microorganisms decomposing the inoculum.

Inoculum of *O. graminis* appeared to decline in natural soil in the same general way with a seedling wheat crop as without one. A similar result has been reported by Tyner (18).

## DISCUSSION

It is realized that the results reported can be considered to apply directly only to the type of inoculum used and to the conditions under which the experiments were conducted. Inoculum in nature is often better protected, may be present in different form, and may occur on and in a variety of substrata. Moreover the environment in the field is quite different from that in a lighted room held at constant temperature. Hence decline of the pathogens concerned, under field conditions, may differ in speed and in other ways; but our own experience and that of others indicates that it does occur and that it is of great significance in agricultural practice.

The reasons for the different rates of decline in the soil of the pathogens studied have not been determined. It is possible that both morphological and physiological factors may be involved. If the former were chiefly responsible, one might expect greater resistance to decline to be shown by *F. culmorum* which forms chlamydospores, next by *H. sativum* which forms thick-walled conidia, and least by *O. graminis* which seldom produces spores in artificial culture. Instead, the positions of *F. culmorum* and *O. graminis* were reversed. A possible morphological explanation of this might lie in the mycelium, as *O. graminis* under some conditions produces dark thick-walled macrophyphae which possibly are more resistant to microbial deterioration than hyphae produced by *F. culmorum* and *H. sativum*. Physiological differences in the pathogens studied are marked and may well include differences in ability to inhibit or otherwise resist antagonistic micro-organisms. It is quite possible too that this ability would vary with the environment. Thus the order of decline of the three pathogens in question might not be the same in two different environments. In this connection it is noteworthy that Rao (14), using an agar plate method for comparing the competitive saprophytic ability of root-infecting fungi, found *F. culmorum* to be a better competitor than either *H. sativum* or *O. graminis*. He points out that his method provides conditions of more severe competition than those obtaining in the soil. Apparently further work will be needed before adequate explanations of decline differences of pathogens in the soil will be forthcoming.

The phenomenon of inoculum decline as a microbiologically-induced decomposition or deterioration process in our opinion should be considered in any accounting of the differences in foot- and root-rot infection obtained in and between sterilized and natural soil. It also should be considered in comparing isolates of the same and different plant pathogens in respect to their ability to produce disease when added to natural soil. Concepts which hold that pathogens are largely indestructible in the soil seem contrary to the growing evidence on hand. Loss of pathogenicity *per se* and fungistatic effects attributed to soil micro-organisms appear inadequate to account wholly for decline.

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# AN EVALUATION OF ALTAI WILD RYE, *ELYMUS ANGUSTUS* TRIN., AS A FORAGE CROP[1]

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## ABSTRACT

A study was conducted with Altai wild rye (*Elymus angustus* Trin.), Russian wild rye (*Elymus junceus*), crested wheatgrass (*Agropyron cristatum*), and intermediate wheatgrass (*Agropyron intermedium*) on their relative merits as forage crops in the arid climate of Saskatchewan. The data show that Altai wild rye produced as much forage as Russian wild rye but less than crested wheatgrass and intermediate wheatgrass. However, it appeared to be less competitive with alfalfa than the other grasses in the test. The nutritive value of Altai wild rye was as good as or better than that of the other grasses; it excelled all of them in crude protein content throughout the season and contained about the same amount of fibre and fat at most stages of development; its ash content was higher than that of the other three grasses in 1954, a wet year, but only higher than the two wheatgrasses in 1957, a very dry year. Comparisons regarding palatability and digestibility suggest that Altai wild rye equals the better grasses in this respect. The study also indicates that nutritive value of grasses throughout the growing season varies much more in wet years when normal plant development occurs, than in dry years when growth is slow and sporadic.

## INTRODUCTION

The search for productive grasses adapted to the arid climatic conditions in the Prairie Plains of Western Canada is a continuing important research problem because, up to the present time, only a few have proved to be useful as cultivated crops. In addition to being winter hardy and drought resistant, it is important that the grasses, which are used for pasture purposes, remain highly nutritive over a long grazing period and especially after maturity when they are considered to be in a cured condition. Pigden (8) described this quality and studied it in a number of native and cultivated grasses. He observed that crested wheatgrass and bromegrass, commonly grown in the Canadian Prairies, did not have as good curing qualities as native grasses and consequently were less nutritious in the summer and fall. Heinrichs and Carson (4), in studying the chemical composition of nine grasses at six stages of development, found significant variations between them for various proximate constituents. Russian wild ryegrass, streambank wheatgrass, and green stipa grass were lowest in lignin content at the mature stage and retained a relatively high fat content at the cured stage. Russian wild ryegrass was especially high in protein content late in the season and was considered to be the most nutritious species in the test and an especially useful one for late season grazing. Stitt (9), in an experiment with seven grass species at Moccasin, Montana, found that Russian wild ryegrass contained more protein than any of the other species and attributed this to its greater leafiness. Bell (2), after analysing numerous species, concluded that about half the grasses studied did not contain sufficient protein to meet the requirements of young growing cattle.

The present study was undertaken to determine growth characteristics

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of a new grass, Altai wild rye (*Elymus angustus* Trin.) (7), compared to crested wheatgrass, Russian wild rye, and intermediate wheatgrass which have been adequately described by Heinrichs and Lawrence (5), Knowles (6), and Heinrichs (3), respectively.

Altai wild rye is a native of Mongolia, has very coarse stems, large seeds, and coarse leaves which grow mainly from the base as in Russian wild ryegrass. It is quite strongly rhizomatic but does not appear to be difficult to eradicate under Saskatchewan conditions. Interest in this grass increased because its chemical composition indicated a high nutritive value and it was found to yield fairly well on dryland at Saskatoon and Swift Current, Saskatchewan. A chemical analysis of 10 plants of each species at the flowering stage from the introduction nursery at Swift Current in 1951 gave these percentage results:

*Altai wild rye* — protein 19.9, ether extract 1.9, crude fibre 28.7, ash 11.3, and N.F.E. 38.1.

*Russian wild rye* — protein 14.7, ether extract 1.7, crude fibre 30.0, ash 8.7, and N.F.E. 44.9.

*Crested wheatgrass* — protein 7.3, ether extract 1.4, crude fibre 32.4, ash 5.1, and N.F.E. 53.8.

## MATERIALS AND METHODS

The species included in the study were:

Altai wild rye — *Elymus angustus* Trin.
Russian wild rye — *Elymus junceus* Fisch.
Crested wheatgrass (Fairway) — *Agropyron cristatum* (L) Gaertn.
Intermediate wheatgrass — *Agropyron intermedium* (Host) Beauv.
Alfalfa (Sc. Ma 501) — a creeping-rooted strain — *Medicago media* Pers.

Two tests were established in May, 1953. Both tests were of the randomized block design with six replications.

### *Test 1*

The grasses were seeded singly in plots consisting of five rows, 35 feet long, and 18 inches apart. The centre row of each plot was used for annual yield determination and the other four for determining chemical composition of the forage throughout the growing season in 2 years. In 1954, the first crop year, cuttings for chemical analyses were made at weekly intervals starting on May 17 and continuing until July 26, a 5-foot length of row being cut on each plot every week. After that, only two further cuttings were made, one on August 10 and one on September 6. Composite samples from the six replicates were analysed for crude protein and crude fibre content and the yield was recorded. Four samples of each species at specific stages of development were analysed for proximate constituents. In 1957, an extremely dry year, samples for chemical analysis were harvested at weekly intervals starting on June 7 and continuing until September 13. In this year samples from each replicate were analysed for proximate constituents separately providing a more accurate measurement of the variables. All the chemical analyses were made by the Chemistry Division, Science Service, Ottawa, employing standard procedures (1).

### *Test 2*

The grasses were seeded in alternate rows with a creeping-rooted strain of alfalfa. The plots consisted of five rows of grass and five rows of alfalfa, 35 feet long and 12 inches apart. The main purpose of the test was to determine the relative competitive ability of the grasses with alfalfa.

TABLE 1. — ANNUAL PRECIPITATION AND EVAPORATION 1953-1957 INCLUSIVE

| Month | 1953 | 1954 | 1955 | 1956 | 1957 | 36-year average |
|---|---|---|---|---|---|---|
| | | | Precipitation in inches | | | |
| January | .93 | .79 | .82 | 1.23 | .56 | .72 |
| February | .95 | .27 | .41 | .67 | .25 | .51 |
| March | 1.50 | .83 | 1.29 | .42 | .76 | .64 |
| April | 1.96 | .93 | 2.76 | .41 | 1.44 | .90 |
| May | 2.35 | 3.37 | 2.58 | 1.24 | .13 | 1.60 |
| June | 3.77 | 3.51 | 1.67 | 3.65 | 1.84 | 2.87 |
| July | .52 | 3.55 | 4.06 | 1.45 | 2.72 | 1.98 |
| August | .51 | 2.77 | .20 | .33 | 1.30 | 1.69 |
| September | 2.06 | 2.74 | .76 | .78 | .35 | 1.18 |
| October | .28 | .41 | .49 | 1.60 | 1.16 | .72 |
| November | .15 | .43 | 1.23 | .23 | .83 | .56 |
| December | .65 | .11 | 1.04 | 1.14 | .46 | .54 |
| Total | 15.63 | 19.61 | 17.31 | 13.15 | 11.80 | 13.91 |
| | | | Evaporation from a free water surface in inches | | | |
| | 26.79 | 21.23 | 25.71 | 28.08 | 31.29 | 28.58 |

In addition to these two field tests, observations on preferential grazing by sheep on the four grasses were made in 1958 and the digestibility of Altai wild rye was determined in a feeding trial using lambs.

The palatability was studied in a test where the grasses occurred in four replicates and on which sheep were allowed free choice grazing from 8.00 a.m. to 5.00 p.m. during two periods, May 21 to June 1, and July 25 to August 12. The observations on palatability were made in three ways: 1) by recording at 5-minute intervals the number of sheep grazing on the various species during the first hour each morning; 2) by the difference method whereby yield on each plot was sampled prior to grazing and immediately after; and 3) by visually rating the intensity of grazing of each grass at the end of the last grazing period.

The digestibility of Altai wild rye was studied in comparison to a standard oat-alfalfa (50:50) hay in a feeding test of the latin square design using 70-pound wethers. The Altai wild rye used for the trial was the aftermath on a seed plot from which the culms and heads had been removed. Thus, it was late season forage consisting mainly of leaves. The feed was cut into inch lengths with a forage cutter and was fed to the natural consumption level of the lambs.

Precipitation records which have a bearing on interpretation of data in this study are presented in Table 1.

## RESULTS

### *Yields*

The dry matter yields of the grasses at the flowering stage are presented in Table 2. The data show that the two wheatgrasses yielded substantially more forage in both tests than either Russian wild rye or Altai wild rye.

TABLE 2. — DRY MATTER YIELDS, 1954-1957 INCLUSIVE

| Grass | Test No. 1 — Grasses seeded alone Grass yield in tons per acre | | | | | Test No. 2 — Grasses seeded in alternate rows with alfalfa Grass plus alfalfa yield in tons per acre | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1954 | 1955 | 1956 | 1957 | 4-year total yield | 1954 | 1955 | 1956 | 1957 | 4-year total yield |
| Altai wild rye | 1.26 | 1.54 | .24 | .31 | 3.35 | 1.27 | 3.01 | .41 | .48 | 5.17 |
| Russian wild rye | 1.50 | 1.04 | .13 | .30 | 2.96 | 1.28 | 3.07 | .30 | .40 | 5.05 |
| Crested wheatgrass | 2.12 | .96 | .26 | .32 | 3.65 | 1.78 | 3.21 | .42 | .47 | 5.89 |
| Intermediate wheatgrass | 2.53 | 1.48 | .19 | .30 | 4.50 | 2.22 | 3.01 | .38 | .52 | 6.13 |
| Mean — 4 grasses | 1.85 | 1.25 | .20 | .31 | 3.62 | 1.64 | 3.07 | .38 | .47 | 5.56 |
| S.E. Mean | .12 | .07 | .01 | .04 | .13 | .06 | .10 | .01 | .03 | .15 |
| S.E. Mean % | 6.49 | 5.60 | 5.00 | 12.90 | 3.67 | 3.54 | 3.32 | 2.10 | 6.38 | 2.70 |

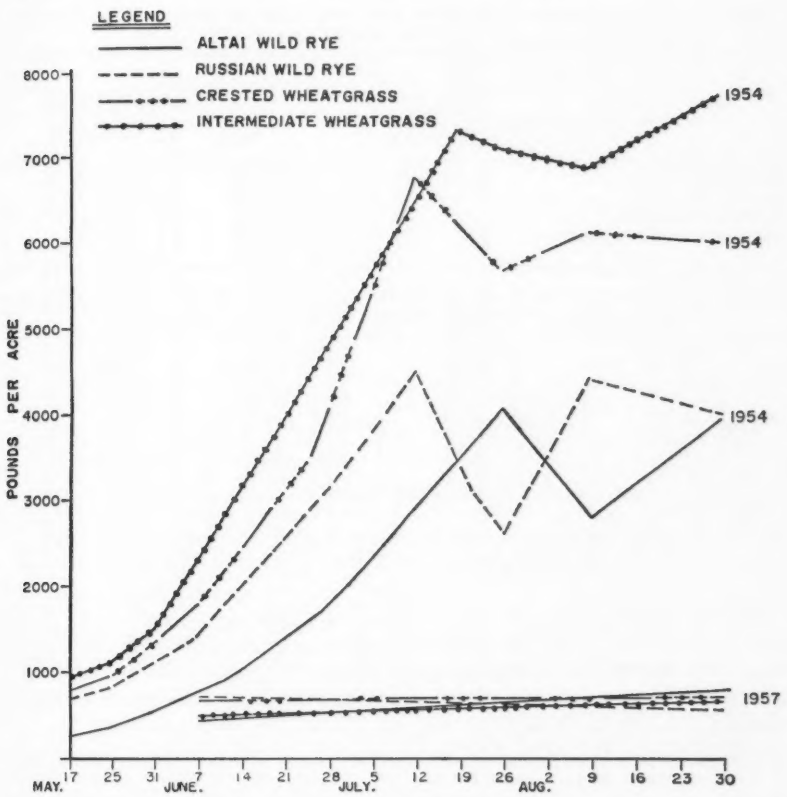


FIGURE 1. Production of four grasses at progressive weekly intervals through the growing season in 1954 and 1957.

The greater yield, however, was most pronounced in the first crop year. The seeding of alfalfa in alternate rows with the grasses had a similar effect on increasing per acre yield with all grasses in all years except the first when Altai wild rye produced an equivalent amount of forage when seeded alone.

Some indication of relative competitive ability of the grasses with alfalfa can be gained by examining the data in Table 3 which presents the per cent of the production contributed by the grass and alfalfa component in Test No. 2. It will be noted that in 1955 the second crop year when precipitation was heavy, all grasses contributed less than 25 per cent to the total production and that the rye grasses contributed less to the yield than the two wheatgrasses. In 1956 and 1957, both dry years, Russian wild rye and crested wheatgrass contributed a much greater percentage to the total forage than either intermediate wheatgrass or Altai wild rye. Since the total production per acre was similar for all species, it would seem to

TABLE 3. — CONTRIBUTION TO THE TOTAL PER ACRE YIELD OF GRASS IN TEST 2, WHERE GRASSES AND ALFALFA WERE SEEDED IN ALTERNATE ROWS

| Grass | % D.M. yield grass | | |
|---|---|---|---|
| | 1955 | 1956 | 1957 |
| Altai wild rye | 13 | 30 | 27 |
| Russian wild rye | 15 | 46 | 65 |
| Crested wheatgrass | 20 | 51 | 55 |
| Intermediate wheatgrass | 25 | 38 | 38 |

TABLE 4. — REGRESSION OF % PROTEIN CONTENT (y) AND % CRUDE FIBRE CONTENT (y) OF FOUR GRASSES ON WEEKLY PROGRESSIVE STAGES OF DEVELOPMENT (x)

| Year | Species | N | Protein | | Crude fibre | |
|---|---|---|---|---|---|---|
| | | | $y^1$ first sampling date | Reg. coeff. b | $y^1$ first sampling date | Reg. coeff. b |
| 1954 | Altai wild rye | 11 | 26.88 | −1.81* | 22.41 | 1.66** |
| | Russian wild rye | 11 | 28.37 | −2.48 | 20.94 | 2.67** |
| | Crested wheatgrass | 11 | 28.09 | −2.63 | 20.93 | 2.07** |
| | Intermediate wheatgrass | 11 | 26.11 | −2.10* | 20.47 | 2.18** |
| 1957 | Altai wild rye | 15 | 10.23 | −.29** | 31.85 | .15** |
| | Russian wild rye | 15 | 10.02 | −.22** | 31.18 | .02** |
| | Crested wheatgrass | 15 | 7.65 | −.30** | 28.27 | .15** |
| | Intermediate wheatgrass | 15 | 8.38 | −.32** | 27.89 | .08** |

*Significant at the 5% point in table of F.
**Significant at the 1% point in table of F.
[1]y calculated from regression equation a + bx.
*Note:* Harvest period at weekly intervals for 1954 was May 17 to July 26, and for 1957 was June 7 to Sept. 13.

be fair to conclude that Russian wild rye and crested wheatgrass offered more competition to alfalfa than the other grasses during the last 2 years of the test.

The growth rates of the four grasses in 1954, the first crop year when normal plant development occurred, and in 1957, a dry year when growth was very sparse, are shown in Figure 1. In 1954 Altai wild rye reached maximum production on July 26, 1 week after intermediate wheatgrass and 2 weeks later than either crested wheatgrass or Russian wild rye. It was also slower to start growth in the spring than the other three grasses. After the peak yield was reached there was some loss in yield, probably attributable to seed shattering and some dropping off of leaves. Later, when new growth appeared, there was an upward yield trend in all four grasses. In 1957, when precipitation fell in negligible amounts throughout most of the season all grasses had produced most of the season's growth by June 7. Russian wild rye actually showed a slight decline in yield from June 7 to August 30, while Altai wild rye showed a slight increase. The yield of the wheatgrasses neither increased nor decreased after June 7. Growth response to light precipitation probably made up for loss of dry leaves.

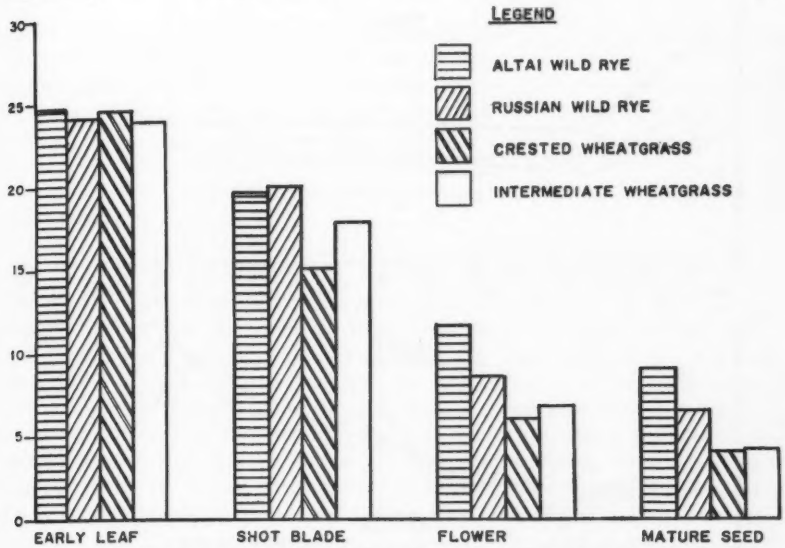


FIGURE 2. The per cent protein content of four grasses at four stages of maturity in 1954, the first crop year when heading of all species was quite profuse.

*Chemical Composition*

The variation in chemical composition of the grasses during the growing season differed greatly between 1954 and 1957. In 1954, the first crop year when moisture supply was adequate, all species developed normally with strong culm formation, while in 1957, an extremely dry season, there was very little additional growth after early June and none of the grasses formed culms. The result was that there was a normal change in nutritive value of the species with the advance of the season in 1954 while in 1957 there was little change throughout the season.

*Protein*

The crude protein content at four specific stages of development for the four grasses in 1954 is shown in Figure 2. The results indicate quite clearly that Altai wild rye contained more protein than the other three grasses at the later stages of development. The regression coefficients presented in Table 4 show the same trend throughout the season of 1954 for Altai wild rye. The reduction in crude protein content per week was considerably lower for it than for the other three grasses. Actually, for both Russian wild rye and crested wheatgrass, the regression coefficient was not significant at the 5 per cent point, indicating that the regression of per cent protein content on progressive weekly stages of development was not linear.

In 1957, a very dry year when none of the four grasses produced culms, the reduction in crude protein content from June 7 to September 13 was very small for all grasses as the regression coefficients in Table 4 indicate. However, in this year the crude protein content was only about one-third as high for all grasses early in June as it was in 1954 when growth was rapid and lush as is indicated by the value of y in the first week of

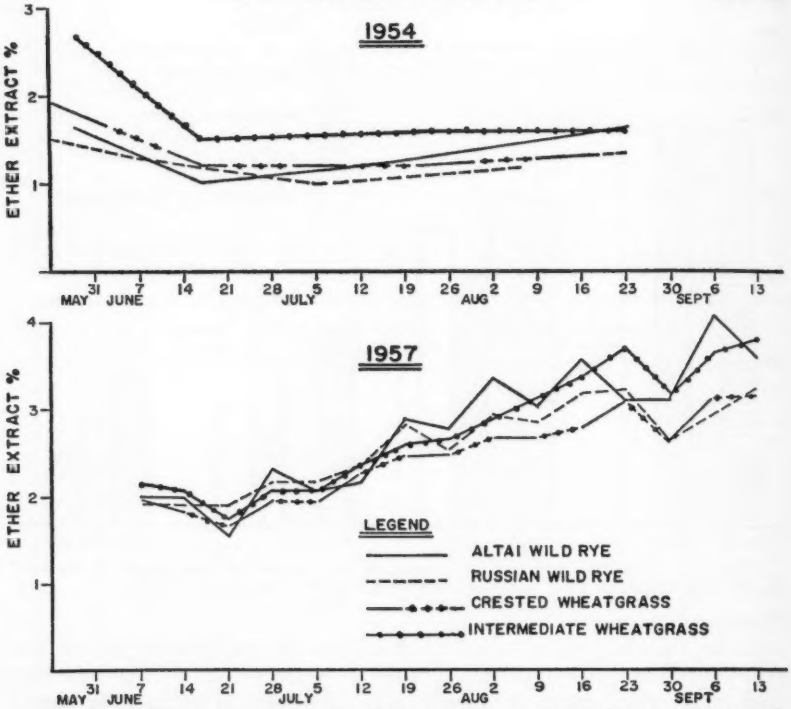


FIGURE 3. Ether extract content of four grasses at progressive periods through the growing season in 1954 (*wet*) and 1957 (*dry*).

the season. This low protein content early in the season was probably due to a considerable amount of dry leaves among the actively growing ones and low available nitrogen in the soil.

*Crude Fibre*

The change in crude fibre content through the growing season is shown in Table 4 by regression coefficients. It will be noted that crude fibre accumulated at a much slower rate in Altai wild rye than in the other three grasses. In Russian wild rye crude fibre content accumulated more rapidly than in crested wheatgrass and intermediate wheatgrass in 1954. This is at variance with data reported previously (4, 5, 8) and probably is due to more profuse culm formation than is usual for this species. The 1957 regression coefficients show that there was very little increase in crude fibre throughout the season and Russian wild rye increased less in this constituent than any of the other species. On July 26, 1954, when all grasses were mature or past maturity, the per cent crude fibre content was: Altai wild rye 39, Russian wild rye 45, crested wheatgrass 39, and intermediate wheatgrass 39. At a comparable time in 1957, when no species formed culms, the respective per cent crude fibre content for the four species was 34, 33, 29, and 30.

*Fat (Ether Extract)*

The trend in fat content of the four grasses throughout the season is

shown graphically in Figure 3. The results show that fat content of the four species, although about at the same level in both 1954 and 1957 early in the season, was much lower after June 15 in 1957, the normal growth year, compared to 1954, the very dry year. In the middle of August the per cent fat content was nearly three times as high in 1957 as in 1954 for all species. The relatively high fat content of Altai wild rye compared favourably with the other three grasses throughout most of the season.

*Ash (Minerals)*

The ash content variation during the growing season is shown graphically in Figure 4 along with the precipitation pattern in 1954 and 1957. The results indicate quite clearly that the ash content of all four grasses declined throughout the season in 1954 when moisture supply was adequate and growth development was normal. On the other hand, in 1957, when the initial moisture supply in the soil was low and the precipitation during the spring and summer came in small amounts, the ash content gradually increased throughout the season. Russian wild rye, which in 1954 contained less ash than either Altai wild rye or intermediate wheatgrass and about as much as crested wheatgrass, contained about twice as much ash as the two wheatgrasses in 1957 and from 50 to 75 per cent more than Altai wild rye. A small amount of green growth developing with showers through the season would seem to have contributed to the increase in ash content of the forage. Russian wild rye, according to the ash content, appears to have responded most in growth to the moderate rainfall in mid-July 1957.

*Palatability*

In Table 5, the data on relative palatability of the four grasses to sheep as observed by three methods are recorded. Russian wild rye was the most acceptable species to sheep. Altai wild rye despite its morphological coarseness appeared to be eaten at least as readily as the two wheatgrasses.

*Digestibility*

A preliminary digestibility trial made with Altai wild rye (Table 6) shows that it compared favourably with oat-alfalfa hay for digestibility and was accepted just as readily. This result was surprising in view of the

TABLE 5. — PALATABILITY OF FOUR GRASSES TO SHEEP UNDER FREE CHOICE GRAZING IN THE SPRING OF 1958, AVERAGE 4 PLOTS

| Grass | A. No. sheep grazing first hour | | | B. % Total yield refused both periods | C. Visual grazing rate[1] |
|---|---|---|---|---|---|
| | May 21 to June 1 | July 25 to Aug. 12 | Total | | |
| Altai wild rye | 11.8 | 39.8 | 51.6 | 39.0 | 2.0 |
| Russian wild rye | 23.4 | 43.0 | 66.4 | 18.4 | 1.0 |
| Crested wheatgrass | 11.8 | 40.6 | 52.4 | 27.3 | 3.2 |
| Intermediate wheatgrass | 15.6 | 48.2 | 63.8 | 51.3 | 3.2 |

[1]Visual grazing rating 1-5 immediately after sheep were taken off the plots in August:
1. Grazed very close to ground
2. Grazed quite intensely
3. Grazed medium
4. Grazed lightly
5. Grazed sparsely — aftermath of 4-6 inches remaining

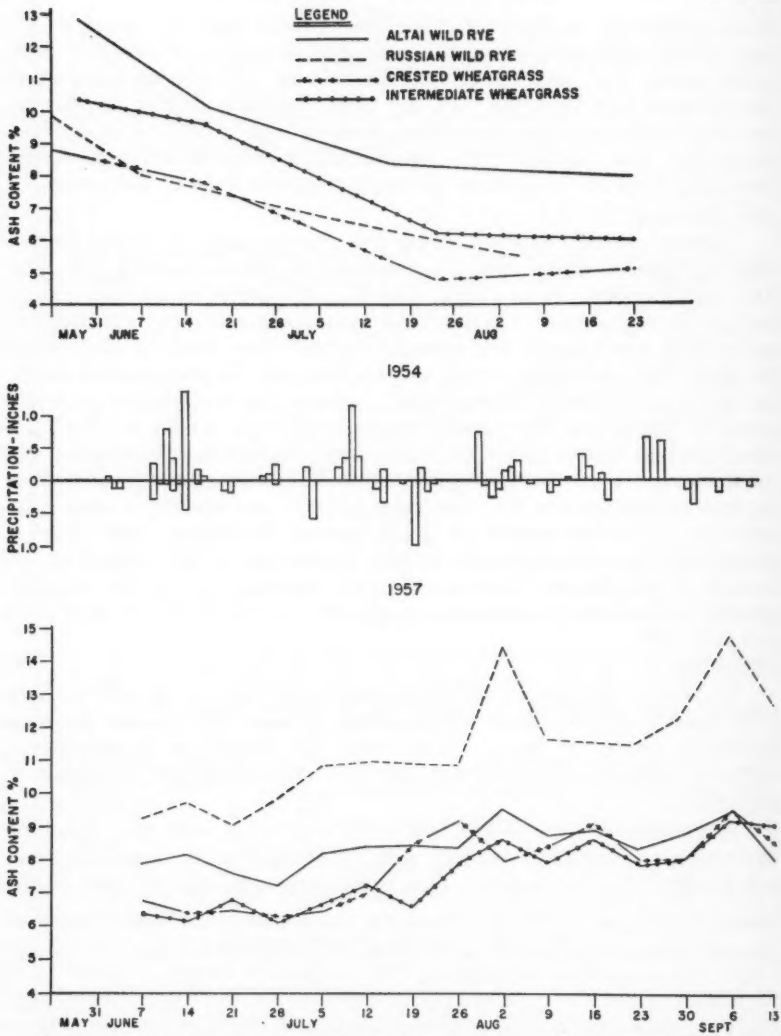


FIGURE 4. Ash content of four grasses through the growing season of 1954 (*above average precipitation*) and 1957 (*far below average precipitation*).

fact that the forage tested represented aftermath from a seed plot and was harvested in August.

## DISCUSSION

Altai wild rye produces about as much forage per season as Russian wild rye, but less than either Fairway crested wheatgrass or intermediate wheatgrass. It is much less competitive with alfalfa than crested wheatgrass and Russian wild rye and also less competitive than intermediate

TABLE 6. — DIGESTIBILITY BY LAMBS OF ALTAI WILD RYE COMPARED TO AN OAT-ALFALFA HAY

| Feed | Dry matter digestibility % | Daily consumption | | Lamb gain lb./day |
|---|---|---|---|---|
| | | D.M. gm./day | D. Energy K. Cals./day | |
| Altai wild rye | 64.5 | 643 | 1759 | .20 |
| 50% oat and 50% alfalfa hay | 65.9 | 609 | 1704 | .15 |

wheatgrass. The nutritive value of Altai wild rye as indicated by proximate constituents appears to be excellent throughout the year. It excels the other three grasses in protein content, especially during the latter part of the season, and its qualities in this respect may render it equal in value to Russian wild rye as a summer and fall pasture grass. The crude fibre content, which one might expect to be high because of the coarse foliage, was about the same as in the other three grasses. The evidence regarding palatability and digestibility suggests that Altai wild rye equals the better grasses in this respect.

The data on chemical constituents point out the great differences in nutritive value that may prevail for the same species in wet and dry years. In a wet year, exemplified by 1954 when culm formation was profuse, there was a much more pronounced decline in nutritive value of the forage through the season than in a dry year, as exemplified by 1957 when no culms developed. Maintenance of a highly nutritious herbage in dry years will be reflected in animal gains and, for a given dry matter intake, animals will do better than in a wet year because of the higher content of such constituents as fat which appreciably affects the energy content of the feed. Another factor which must be considered when studying the nutritive value of grasses is the decline of available nitrogen in the soil as the age of the grass stand increases, with the result that protein content may be abnormally low in many species causing stunted growth and no culm formation. Heinrichs and Carson (4) found the protein content of nine grasses to be much lower in the second crop year than in the first. The rather low protein content early in the season of herbage in 1957 compared to 1954 in this study may have been partly due to low available nitrogen in the soil which limited growth.

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# DIFFERENCES IN INTENSITY OF UNRIPE FRUIT COLOUR IN THE TOMATO[1]

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**ABSTRACT**

Differences in the intensity and pattern of the mature-green fruit colour of five tomato varieties and their hybrids were evaluated visually and with a colour reflectant-meter (Photovolt). The differences were greater than could be explained on the basis of the two known non-allelic genes u and ug. The results suggest the existence of either an allele for u or ug or, more likely, a third gene that influences the intensity of green fruit colour in the tomato.

## INTRODUCTION

In the tomato, varietal differences in the intensity and pattern of the mature-green colour have been attributed to two non-allelic genetic factors. The observations to be presented suggest that other genes, or alleles of the known genes, exist that affect the green colour of the tomato fruit.

MacArthur (2, 3), who first described the uniform green of unripe tomato fruits as contrasted to the dark green stem-end, attributed it to the recessive genetic factor *u* on chromosome 7 (now chromosome 10, linkage group VII). Bohn and Scott (1) obtained evidence in the variety Uniform Globe of a second recessive gene $u_2$ (now *ug*) for uniformity of colour, which was on a different chromosome from *u*. In crosses of Danmark *uu UgUg* with Uniform Globe *UU ugug* they obtained fruit with dark green stem-ends in the $F_1$ and a segregation of 9 dark green stem-end to 7 uniform green in the $F_2$. These results were substantiated and refined by Reynard (5), who obtained a 9:3:4 $F_2$ segregation of dark green stem-end, uniform ripening, and grey uniform ripening, the latter two phenotypes being difficult to differentiate.

The economic value of the uniform fruit colour in the promotion of earlier maturity was recognized by Yeager (6). Evidence of linkage between resistance to radial fruit cracking and the pattern of uniform ripening dependent upon the gene pair *ugug* was obtained by Reynard (4). Further evidence of linkage of resistance to radial, concentric, and burst cracking with several characters including uniformity of colour has been reported by Young (7).

Differences in the intensity of the uniform green colour exist between two tomato varieties, Early Lethbridge and Earlinorth, developed by the Lethbridge Research Station from a single cross of Farthest North x Polar Circle. A comparison of the uniformly coloured varieties Danmark, Early Lethbridge, Uniform Globe, and Earlinorth indicated that they differed, becoming progressively lighter in the order mentioned. Evaluations of differences in the colour intensity of these varieties and their hybrids are reported.

## MATERIALS AND METHODS

The uniformly green tomato varieties Danmark (*uu UgUg*), Earlinorth (?), Early Lethbridge (?), and Uniform Globe (*UU ugug*), plus a dark green stem-end variety Firesteel (*UU UgUg*) were crossed in all combinations including the reciprocals. Three $F_1$ plants of each cross and their parents were grown in the greenhouse during the winter, when the intensity

[1]Contribution from the Horticulture Section.

of sunlight was not sufficient to affect the colour differences. Colour evaluation of the fruit was made at the mature-green stage.

To avoid discrepancies in visual colour evaluation a reflectance-meter (Photovolt Model 610) was employed to measure differences in colour. Tri-stimulus filters were used in measuring the stem-end, side, and blossom-end of individual fruits of each parent and $F_1$ plant. Only the stem-end readings proved to be of value in determining colour differences. Although the reciprocal of each cross was examined, no variation due to cytoplasmic inheritance was detected. The reflectance-meter readings for the stem-end, based on six fruits per variety or hybrid, were averaged and arranged in ascending order. In addition, notes were made of the visual colour pattern variations of each variety and hybrid.

## RESULTS

The intensity of colour in the varieties and hybrids resulted in a definite pattern of response on the reflectance-meter scale, as shown in Table 1. The notes made of the visual colour pattern differences were found to agree well with the trend established from the reflectance readings. The discrepancies that occurred in the reflectance readings in Table 1 with respect to the sequence of the varieties and hybrids were generally below 0.5 and never more than 2.0. In instances in which a discrepancy was greater than 0.5, the reading obtained for the other two filters was considered sufficient to warrant the position given the hybrid with respect to that immediately above it.

The location of Uniform Globe with respect to the other lines may not be entirely accurate owing to the presence in this variety of the recessive gene pair *yy* for colourless skin. The skin colour of the other varieties is yellow *YY*.

The cross of Danmark x Uniform Globe produced $F_1$ fruit with dark green stem-ends, as reported by Bohn and Scott (1) and Reynard (5). Instrument readings of the crosses (Table 1) involving Earlinorth x Danmark and Early Lethbridge x Danmark were high, indicating a light colour. Visual observations of the two crosses indicated a uniform light or pale green colour, which agreed with the instrument determinations. Thus it would seem that neither of the Lethbridge varieties is similar to Uniform Globe for green fruit colour.

On the other hand, the $F_1$ fruits of Earlinorth x Uniform Globe had instrument readings similar to those of Danmark x Uniform Globe. The visual colour classification (Table 1) also suggests a similarity between these two hybrids, as they were the only two dark green stem-end lines without the variety Firesteel in their parentages. These hybrid combinations suggest that Earlinorth and Danmark possess the same genotype for green fruit colour, which complements that of Uniform Globe to produce the dark green stem-end. However, both the instrument determinations and visual colour pattern indicate that Earlinorth and Danmark differ, as Earlinorth is much lighter green than Danmark.

The differences between Early Lethbridge and Earlinorth or Danmark are apparent (Table 1) from the cross Early Lethbridge x Uniform Globe, in which the $F_1$ fruit is uniform green with no trace of the dark green stem-

TABLE 1.—REFLECTANCE-METER DETERMINATIONS OF THE GREEN FRUIT COLOUR PATTERN OF FIVE TOMATO VARIETIES AND THEIR $F_1$ HYBRIDS BASED ON THE AVERAGE OF SIX FRUITS PER VARIETY OR HYBRID

| Variety or hybrid | Colour filter (coloured reflected) | | | Observed colour pattern |
|---|---|---|---|---|
| | Blue (yellow) | Green (red) | Amber (green) | |
| Firesteel | 5.5000 | 15.2000 | 13.6000 | Dark green/medium light green |
| Firesteel x Uniform Globe | 6.5707 | 18.7500 | 16.7500 | Medium dark green/medium green |
| Firesteel x Early Lethbridge | 6.5416* | 19.3333 | 18.1666 | Dark green/medium green |
| Firesteel x Danmark | 6.7500 | 18.5000** | 18.7500 | Medium dark green/pale green |
| Firesteel x Earlinorth | 6.6666* | 20.7500 | 19.0000 | Medium dark green/medium pale green |
| Earlinorth x Uniform Globe | 7.5104 | 24.8542 | 22.4791 | Medium dark green/pale green |
| Danmark x Uniform Globe | 10.0833 | 28.2916 | 26.8125 | Medium light green/pale green |
| Danmark | 10.1667 | 27.0000** | 26.5000* | Medium green |
| Early Lethbridge x Uniform Globe | 9.7600* | 30.8375 | 27.3333 | Light medium green |
| Early Lethbridge x Earlinorth | 9.8750* | 31.1250 | 31.0625 | Light medium green |
| Early Lethbridge | 11.5625 | 31.2500 | 31.0000** | Pale green |
| Early Lethbridge x Danmark | 12.5313 | 32.8333 | 32.2084 | Pale green |
| Earlinorth x Danmark | 12.6250 | 33.6709 | 30.7500 | White green |
| Uniform Globe | 13.1668 | 34.3333 | 33.3333 | Grey green |
| Earlinorth | 13.9167 | 35.0000 | 31.5000** | White green |

* Discrepancy $< 0.5$
** Discrepancy $> 0.5 < 2.0$

end that occurs in crosses of Earlinorth or Danmark with Uniform Globe. Furthermore, the instrument colour determinations indicate that the intensity of colour of Early Lethbridge fruit is midway between those of Danmark and Earlinorth.

Differences in the intensity of the dark green stem-end are evident (Table 1) in those crosses in which Firesteel is the common parent. The reflectance-meter readings, especially with the green and amber tri-stimulus filters, indicate that the $F_1$ fruit was lighter than the variety Firesteel and that the intensity differed between the Firesteel hybrids. The visual colour evaluations of these crosses were not in as close agreement with the instrument readings as were those of the uniformly green material. It was difficult, therefore, to determine to what extent these various crosses involving Firesteel actually differed.

## CONCLUSIONS

Reflectance-meter determinations and visual colour evaluation of five tomato varieties and their $F_1$ hybrids indicate that the varieties differ in the intensity and pattern of green fruit colour. The differences between the dark green stem-end and uniformly green fruit are due to at least two non-allelic genes, *U* and *Ug*, which complement one another to produce the dark green stem-end. These two genes individually produce uniform green fruit of different intensities; however, they do not adequately explain the differences observed between the varieties Danmark, Earlinorth, Early Lethbridge, and Uniform Globe. In addition, the differences that appear to exist in the various crosses with Firesteel cannot be explained on the basis of these two genes alone.

The observations suggest that Earlinorth, like Danmark, possesses the *Ug* plus another gene affecting colour intensity, rather than allele of *Ug*. An allele of *Ug* would be unlikely as it would be required to have a similar complementary action with *Ug* plus the ability to form a different intensity of uniform colour. Early Lethbridge, which arose from the same cross as Earlinorth, does not possess either *U* or an allele. This is indicated by its failure to produce a dark green stem-end in combination with *Ug*. The same applies for any alleles of *Ug* that Early Lethbridge might have. This further suggests the existence of another gene for green colour such as might be possessed by Earlinorth but acting alone without *U* in Early Lethbridge. This investigation is being continued with $F_2$ and backcross populations in an effort to further evaluate these observations.

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# INFLUENCE OF CULTURAL PRACTICES ON INCIDENCE OF COMMON ROOTROT IN WHEAT IN SASKATCHEWAN[1]

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**ABSTRACT**

Mouldboard ploughing of stubble land in preparation for a second consecutive wheat crop was compared with surface tillage for effect on common rootrot and yield in a study at the Experimental Farm, Swift Current, Sask., during the years 1951-58. Ploughing was found to be effective in reducing the incidence of rootrot in the seedling stage. As the season advanced increasing numbers of plants became infected and by maturity of the crop differences between ploughing and surface tillage frequently had disappeared. In general, yields were not significantly increased by ploughing.

## INTRODUCTION

*Helminthosporium sativum* P. K. and B. is the dominant pathogen in the common rootrot complex on the Canadian Prairies. Species of *Fusarium*, particularly *F. culmorum* (W. G. Sm.) Sacc., are also of importance as indicated in studies by Broadfoot (1) and Tyner (11). *F. culmorum* is a soil inhabitant, whereas *H. sativum* is a root inhabiting fungus (4); however, both fungi sporulate freely on crowns and other invaded tissue of host plants. Although *H. sativum* apparently does not exist saprophytically in soil a high percentage of the spores remain viable though dormant for up to 2 years under field conditions (3). Because crop residues remain on or near the surface, the spores too are mainly in the surface soil. Simmonds *et al.* (10) refer to the occurrence and distribution of *H. sativum* in Saskatchewan and Ledingham and Chinn (7) report briefly on the numbers of spores of the fungus in some Saskatchewan fields.

In view of a growing realization that spores of *H. sativum* were the important source of infection, and that for the most part they were confined to surface layers of soil, it seemed worthwhile to determine the effect on common rootrot of turning under spore infested surface soil and crop residues with the mouldboard plough.

Although tillage in Saskatchewan is mainly accomplished with the one-way disk, the heavy duty cultivator, or other implements that retain crop residues in the surface layers of soil, there are a few areas in which the mouldboard plough is still regarded favourably in preparation of stubble land for a second crop. This operation is normally done in the spring, followed immediately by seeding, usually with a press-drill. Advantage was taken of an experiment in progress at the Experimental Farm, Swift Current, Saskatchewan, involving various cultural treatments of stubble land prior to planting a second consecutive crop of wheat. The primary object of the study was to determine whether crop sanitation, as effected by use of the mouldboard plough, would have a significant influence on rootrot in the subsequent wheat crop. While the study was mainly con-

[1]Joint contribution from Canada Department of Agriculture Research Station, Saskatoon, Sask., (No. 27), and Experimental Farm, Swift Current, Sask.

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cerned with *H. sativum* it is thought that the same crop sanitation measures would apply also to *F. culmorum*. In this connection, Hall (5), in a study covering one crop year in Oregon, reported that mouldboard ploughing as compared to disk and sweep ploughing did not reduce the incidence or severity of "brown root-rot" caused by *Fusarium roseum* f. *cerealis* (Cke) emend Snyd. & Hans.

## METHODS

The experiment on stubble treatments at Swift Current was started in 1946. It comprised three 6-acre blocks of Haverhill-Wood Mountain clay loam soil in a summerfallow–wheat–wheat rotation. The cultural treatments were carried out each year on wheat stubble in preparation for the second crop of wheat. The treatments originally were as follows: 1) plough in the spring, pack and seed with a press-drill; 2) burn the stubble, work with a cultivator and seed with a press-drill; 3) one-way disk the land and seed with a press-drill; and 4) one-way disk and seed in the same operation. These methods were modified later, as indicated in Table 1. The treatments were replicated four times in randomized blocks. In each plot, which consisted of about two-thirds of an acre, one-half acre was harvested by taking two widths of the combine for the full length of the plot. The different tillage operations loosened the soil to varying depths, but care was taken to seed at a uniform depth of 1½ to 2 inches throughout the experiments. The depth of regular ploughing was about 5 inches and of shallow ploughing about 3 inches. In general, crop residues and surface soil were well buried by the plough; inevitably, though, a narrow band occurred along the upper edge of each furrow where turning under of surface material was incomplete.

Rescue wheat was used until 1954, when it was replaced by the variety Chinook. Both varieties are classed as moderately susceptible to common rootrot. Data on common rootrot were taken in the years 1951-58, inclusive. Records were made three times each summer: late seedling stage; soon after heading; and at or slightly before maturity. Each plot was sampled by two persons, each of whom took a total of 100 plants at random in groups of 4 or 5 at 5- to 10-yard intervals. In the seedling stage the number of plants showing lesions on the coleoptile was recorded as a percentage of the total number examined. In the two later stages of crop development the plants were grouped into three classes – slight, moderate and severe, depending on extent and severity of lesions on the crown and subcrown internode. To obtain the disease rating in per cent, the numbers of plants in the slight, moderate and severe groups were multiplied by factors of 2, 5 and 10, respectively; the products were then summed and converted to a basis of 100 plants. Thus, the data for the seedling stage simply record the percentage of visibly infected plants, whereas the data for the second and third samplings are intended to measure both the incidence and severity of disease.

## RESULTS

Data obtained over the 8-year period 1951-1958 are presented in Table 1. The disease ratings showed considerable variation from year to

TABLE 1.—INFECTION OF WHEAT BY COMMON ROOTROT AT THREE STAGES OF GROWTH, AND YIELDS ON STUBBLE LAND CULTIVATED IN VARIOUS WAYS. SWIFT CURRENT, SASK., 1951-58

| Year | Ploughed, packed, and seeded with press-drill | Seeded with one-way disk | Burned, cultivated, seeded with press-drill[1] | Worked with one-way disk, seeded with press-drill[2] | Differences required for significance at | |
|---|---|---|---|---|---|---|
| | | | | | 5% level | 1% level |
| | | | Seedling stage; percentage infected | | | |
| 1951 | 28 | 81 | 65 | 69 | 11 | 15 |
| 1952 | 79 | 93 | 90 | 91 | 10 | 14 |
| 1953 | 54 | 64 | 47 | 46 | 12 | 17 |
| 1954[4] | 7.7 | 13.6 | 13.3 | 12.9 | 4.4 | 6.4 |
| 1955 | 23 | 36 | 34 | 32 | 10 | 15 |
| 1956 | 35 | 67 | 66 | 52 | 17 | 24 |
| 1957 | 26 | 35 | 33 | 41 | 7 | 11 |
| 1958 | 11 | 49 | 29 | 79 | 20 | 29 |
| 7 yr. av. | 37 | 61 | 52 | 59 | | |
| | | | Heading stage; disease rating[3] | | | |
| 1951 | 11.9 | 19.5 | 22.7 | 11.5 | 3.5 | 5.0 |
| 1952 | 10.8 | 13.8 | 12.2 | 12.6 | 2.2 | 3.1 |
| 1953 | 10.6 | 9.3 | 7.4 | 6.9 | — | — |
| 1954 | 15.8 | 19.7 | 17.3 | 19.6 | — | — |
| 1955 | 5.8 | 12.2 | 7.0 | 6.0 | 3.6 | 5.2 |
| 1956 | — | — | — | — | — | — |
| 1957 | 9.8 | 12.4 | 11.9 | 11.6 | — | — |
| 1958 | 8.7 | 18.4 | 10.4 | 20.3 | 2.4 | 3.4 |
| 7 yr. av. | 10.5 | 15.0 | 12.7 | 12.6 | | |

TABLE 1.—INFECTION OF WHEAT BY COMMON ROOTROT AT THREE STAGES OF GROWTH, AND YIELDS ON STUBBLE LAND CULTIVATED IN VARIOUS WAYS. SWIFT CURRENT, SASK., 1951-58—*CONTINUED*

| Year | Ploughed, packed, and seeded with press-drill | Seeded with one-way disk | Burned, cultivated, seeded with press-drill[1] | Worked with one-way disk, seeded with press-drill[2] | Differences required for significance at | |
|---|---|---|---|---|---|---|
| | | | | | 5% level | 1% level |
| | | | Mature crop; disease rating | | | |
| 1951 | 17.7 | 29.4 | 29.5 | 20.8 | 5.8 | 8.3 |
| 1952 | 9.9 | 20.1 | 17.3 | 12.4 | 1.8 | 2.5 |
| 1953 | 13.1 | 16.9 | 11.7 | 12.1 | — | — |
| 1954 | 40.6 | 38.7 | 32.9 | 30.8 | — | — |
| 1955 | 7.3 | 9.6 | 9.0 | 8.0 | 1.8 | 2.6 |
| 1956 | 6.4 | 7.4 | 7.2 | 9.2 | — | — |
| 1957 | 13.2 | 19.1 | 16.8 | 14.0 | — | — |
| 1958 | 13.0 | 27.3 | 12.2 | 27.5 | 8.2 | 11.8 |
| 8 yr. av. | 15.1 | 21.5 | 17.1 | 16.8 | | |
| | | | Yield of grain (bu. per acre) | | | |
| 1951 | 13.0 | 14.0 | 13.8 | 13.3 | — | — |
| 1952 | 36.6 | 35.7 | 37.5 | 35.6 | — | — |
| 1953 | 18.9 | 18.6 | 19.8 | 19.7 | — | — |
| 1954 | 21.0 | 19.2 | 21.2 | 21.6 | — | — |
| 1955 | 25.9 | 22.9 | 23.4 | 22.7 | 1.3 | 1.9 |
| 1956 | 20.9 | 20.7 | 19.5 | 19.7 | — | — |
| 1957 | 9.3 | 8.0 | 8.4 | 7.6 | 0.9 | 1.3 |
| 1958 | 8.9 | 9.1 | 9.2 | 9.2 | — | — |
| 8 yr. av. | 19.3 | 18.5 | 19.1 | 17.8 | | |

1 Starting in 1953, this treatment was replaced by deep tillage in the fall with the heavy duty cultivator and spring seeding with the press-drill; in 1958 the heavy duty cultivator was dropped in favour of shallow spring ploughing followed by seeding with the press-drill.
2 In 1953 this treatment was replaced by fall tillage with the Noble-blade and spring seeding with the press-drill; in 1957 spring cultivation with the roto- tiller replaced the Noble-blade.
3 Method of computing disease rating: plants with slight, moderate, or severe lesions on crown and subcrown internode were grouped and the number in each group multiplied by factors of 2, 5 and 10, respectively; the products were then converted to a basis of 100 plants.
4 Disease ratings, rather than percentages infected plants.

year and from one sampling to the next; however, there were well defined trends. In the seedling stage the percentages of infected wheat seedlings on ploughed land were, almost without exception, lower than in the surface-type cultural treatments. At the heading stage ploughing presumably was responsible for reduced disease in 4 out of 7 years. Data for this stage of development were not obtained in 1956. At crop maturity significantly lower disease was observed in the ploughing in only 4 years of the 8. These 4 years were also ones in which significance was noted at the second date of sampling. It is noteworthy that burning of the stubble prior to seeding in 1951 and 1952 did not lower the incidence of rootrot.

Yield differences were either very slight or non-existent. The data show no correlation between yield and incidence of rootrot. The years 1951, 1952, and 1958 were marked by significantly less rootrot in the ploughing than in other treatments at all three dates of sampling; yet in no one of these years was the yield from ploughed plots higher than from other cultural treatments. The only years when yields on ploughing were significantly higher than in the other treatments were 1955 and 1957. In 1955, disease ratings at maturity of the crop were only slightly lower on ploughed than on surface worked land and in 1957 there were no significant differences.

## DISCUSSION

In this experiment the comparison essentially is ploughing vs. surface cultivation methods that maintain a trash cover. Ploughing as a method of rootrot control is based on the premise that spores and other inocula of *H. sativum* which are largely produced near the soil surface will be buried below the seeding level and thus the seedlings tend to escape infection. It is apparent though that the roots of the growing crop must penetrate the spore-infested soil turned under by the plough. There is some evidence, however, that this may not be a serious consideration. Chinn* found that little root infection occurred in wheat seedlings growing through spore-infested soil. Moreover, Chinn and Ledingham (2) reported that wheat roots do not stimulate germination of *H. sativum* spores in soil.

There were no marked differences in rootrot among the various surface tillage methods. Ploughing, however, reduced the incidence of disease, particularly in the seedling stage, but the differences lessened as the season advanced. Additional records on a number of farmers' fields in 1953 showed conclusively the lower incidence of common rootrot infections in the seedling stage on ploughed fields as compared to those that had been surface worked. Also a number of the earlier publications relating to common rootrot list summerfallow as a control measure. Henry (6) and Broadfoot (1) both reported less common rootrot in summerfallow than in stubble wheat. Summerfallowing prior to the early 1930's, including the period covered by these earlier studies, implied the use of the mouldboard plough. Thus it is likely that the observed control was due mainly to burial of spores and infected crop residues. In more recent years crop surveys have shown no marked differences in rootrot between stubble and summerfallow wheat. In 1952, records of a survey in the three Prairie

*Unpublished data.

Provinces (8) showed 192 summerfallow fields with an average disease rating of 7.2 and 57 stubble fields with an average of 6.3. A comparable survey carried out in 1958 (9) gave an average rating of 9.0 on 190 summerfallow wheat fields and a rating of 10.1 on 52 stubble fields. The similarity of disease rating on summerfallow and stubble wheat is thought to be due to the fact that present-day summerfallow seldom entails the use of the mouldboard plough.

The tendency for rootrot infection of wheat on ploughed land to "catch-up" as the season advances to the initially higher infections of surface-worked fields is due, no doubt, to a number of factors. Wind dissemination of spores may account for some re-infestation of the surface soil and bring about infections. Spore production during the growing season from early infections of the crop may provide fresh inocula. Rain is probably effective in moving spores about on the soil surface, thus bringing them into proximity with a host so that infection may occur. The end result of the "catch-up" phenomenon is a lessening of the differences between ploughing and surface tillage to a point where, generally speaking, the practicability of ploughing appears questionable.

Significantly higher yields were obtained from the ploughed land in only 2 years out of 8. Nevertheless, it would be incorrect to assume that common rootrot is therefore of little or no consequence in crop production. The differences in disease ratings between ploughing and surface tillage at maturity of the crop were slight. Furthermore, in considering yields it should be kept in mind that moisture is almost always the main limiting factor, and this is particularly so in the case of stubble crops, with which this study deals. Also the experiment was on land that had very few weeds; thus competition to rootrot weakened plants was provided by other wheat plants rather than by weeds. With these factors in mind it is considered that only wide differences in disease rating, in vigour and even in numbers of plants would be reflected in difference in yield.

## ACKNOWLEDGEMENTS

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# EFFECTS OF CUTTING TREATMENTS ON LADINO CLOVER GROWN ALONE AND IN MIXTURE WITH GRASSES
## I. PRODUCTIVITY AND BOTANICAL COMPOSITION OF FORAGE[1]

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**ABSTRACT**

Ladino clover, grown alone and in mixture with timothy and smooth bromegrass, was cut two, four and six times per season to a height of 1½ and 3 inches. Forage yields were collected in 1955 and 1956 from a seeding made in 1954 at the Experimental Farm, Lennoxville, Quebec.

Ladino clover yielded more clover when grown alone than when associated with a grass. The mixtures did not differ in clover yields. In the grass fraction, timothy outyielded bromegrass in 1955 only. Mixtures produced more total forage than Ladino clover alone. Cutting to 1½ inches gave higher fraction and total yields during both years than cutting to 3 inches, except in the grass fraction in 1956. The mixtures yielded more clover but much less grass and total production when cut four times than when cut twice. The lowest yields occurred with six cuttings. With Ladino clover alone, increased frequency of cutting depressed yields. The mixed swards contained the most clover when cut four times to 1½ inches and the least when cut twice to 3 inches.

### INTRODUCTION

Although Ladino clover has been known in Quebec for several decades, it was not until the late '40's that it occupied a place on farms. Since then it has spread rapidly and has become one of the most valuable pasture species.

The many desirable qualities and the adaptability of Ladino clover to varying soil and climatic conditions led to its wide acceptance. Fundamental data, however, with respect to its management under Quebec conditions are very limited. Investigations, therefore, were undertaken on seeding mixtures and cutting treatments in order to obtain such information on the culture of Ladino clover. These studies were carried out during 1955 and 1956 at the Experimental Farm, Lennoxville, Quebec.

### REVIEW OF LITERATURE

Studies by Brown and Munsell (1), Wagner (6), Tesar and Ahlgren (5), Jeffers (3) and others showed that mixtures of Ladino clover with certain grasses were more productive than Ladino clover alone. Moreover, less heaving during the winter was recorded (1) and weeds were reduced during the growing season (6) when Ladino clover was grown in mixed swards.

Several workers have studied the effect of managerial treatments on the productive life of Ladino clover. Brown and Munsell (2) found that mowing Ladino clover-grass mixtures 2 inches above the ground resulted in much better stands of clover and larger yields of dry matter than cutting to 4 inches. For similar reasons, Sprague and Garber (4) favoured a 2-inch to a 3-inch height of cut. Jeffers (3) reported that cutting Ladino clover grown alone or in mixtures to a height of 1 inch resulted in higher

[1]Part of a thesis submitted in partial fulfilment of the requirements of the degree of Doctor of Philosophy, University of Wisconsin. 1958.
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yields of forage than mowing to a height of 3½ inches irrespective of the number of cuttings. He also obtained the year following seeding significantly higher yields with four cuttings than with either two or six cuttings. However, during the second harvest year, the Ladino clover-grass mixtures cut four times did not differ significantly in productivity from those cut twice. Tesar and Ahlgren (5) concluded, from their 2-year study on the management of Ladino clover, that cutting four times to a height of 3½ inches appeared to be the most satisfactory treatment to maintain a high production over a period of years.

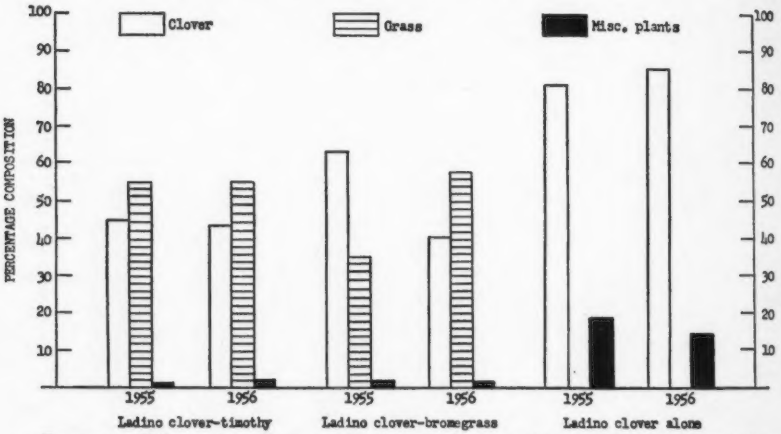


FIGURE 1. Effect of seedings on the botanical composition of forage harvested in 1955 and 1956.

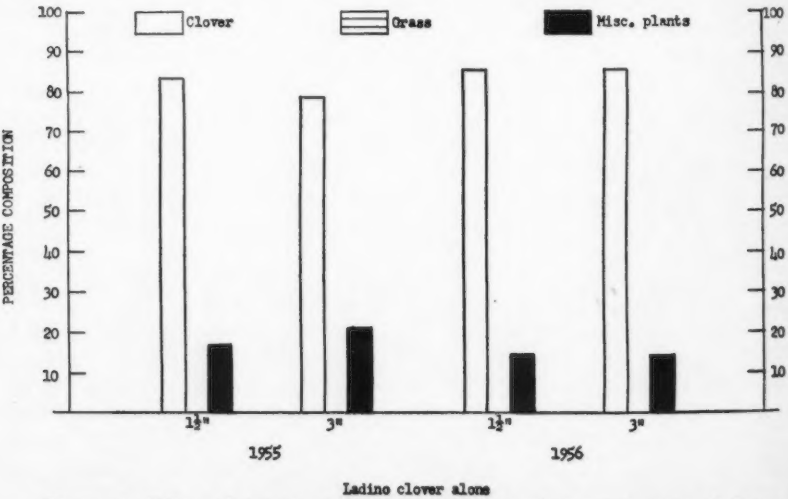


FIGURE 2. Effect of height of cutting on the botanical composition of forage harvested in 1955 and 1956 from Ladino clover grown alone.

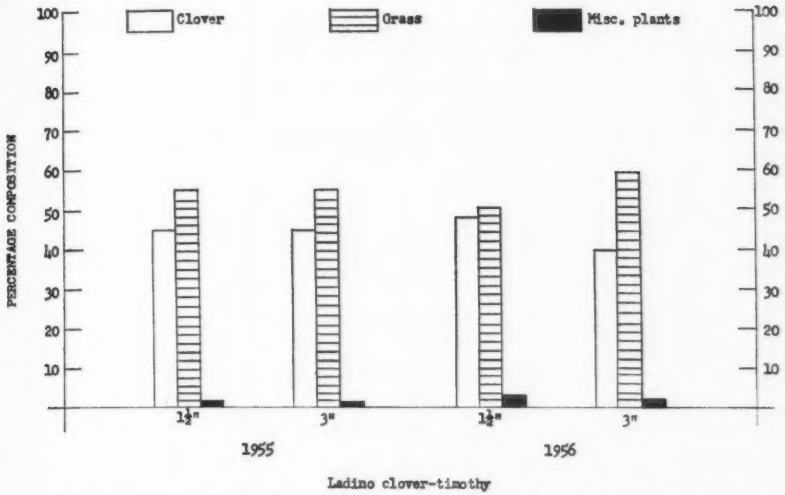


FIGURE 3. Effect of height of cutting on the botanical composition of forage harvested in 1955 and 1956 from a Ladino clover-timothy mixture.

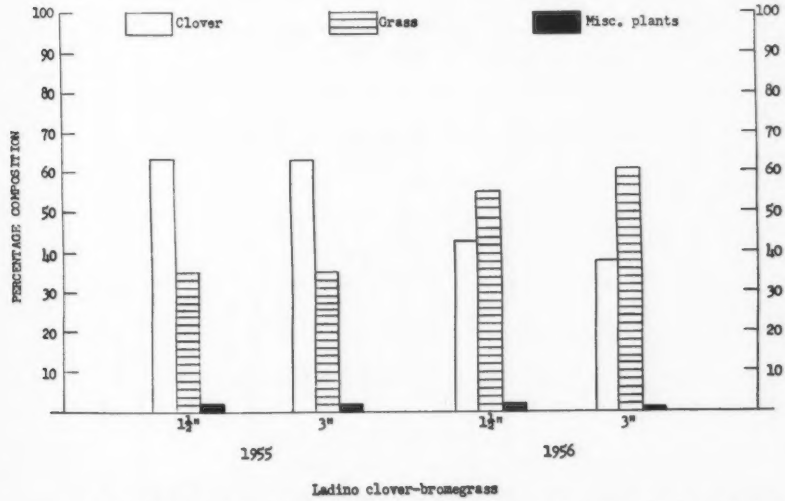


FIGURE 4. Effect of height of cutting on the botanical composition of forage harvested in 1955 and 1956 from a Ladino clover-bromegrass mixture.

## MATERIALS AND METHODS

The investigations were conducted on a Coaticook silt loam characterized by good surface drainage and moderate fertility. The pH of the soil was 5.7. Two tons of finely-ground limestone were applied per acre prior to seeding and worked in by several diskings. An application of

TABLE 1. — EFFECT OF SEEDINGS ON THE FRACTION AND TOTAL YIELDS OF DRY MATTER IN POUNDS PER ACRE FOR EACH HARVEST YEAR

| Seeding | Clover fraction | | | Grass fraction | | | Total production | | |
|---|---|---|---|---|---|---|---|---|---|
| | 1955 | 1956 | Average | 1955 | 1956 | Average | 1955 | 1956 | Average |
| Ladino clover alone | 1841 | 2076 | 1959 | — | — | — | 1841 | 2076 | 1959 |
| Ladino clover — timothy | 1490 | 1341 | 1415 | 1943 | 1782 | 1863 | 3433 | 3123 | 3278 |
| Ladino clover — bromegrass | 1648 | 1172 | 1410 | 960 | 1940 | 1450 | 2608 | 3112 | 2860 |
| L.S.D. — All three seedings 5% | N.S. | 158 | 242 | — | — | — | 428 | 311 | 238 |
| L.S.D. — Two mixtures 5% | N.S. | N.S. | N.S. | 142 | N.S. | 143 | 766 | N.S. | 368 |

TABLE 2. — EFFECT OF HEIGHT OF CUTTING ON THE FRACTION AND TOTAL YIELDS OF DRY MATTER IN POUNDS PER ACRE FOR EACH SEEDING AND EACH HARVEST YEAR

| Seeding | Height of cutting | Clover fraction | | | Grass fraction | | | Total production | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1955 | 1956 | Average | 1955 | 1956 | Average | 1955 | 1956 | Average |
| Ladino clover alone | 1½″ | 2189 | 2428 | 2309 | — | — | — | 2189 | 2428 | 2309 |
| | 3″ | 1493 | 1725 | 1609 | — | — | — | 1493 | 1725 | 1609 |
| Ladino clover — timothy | 1½″ | 1655 | 1614 | 1635 | 2186 | 1763 | 1974 | 3841 | 3377 | 3609 |
| | 3″ | 1324 | 1068 | 1196 | 1700 | 1802 | 1751 | 3024 | 2870 | 2947 |
| Ladino clover — bromegrass | 1½″ | 1883 | 1299 | 1591 | 1110 | 1954 | 1532 | 2993 | 3253 | 3123 |
| | 3″ | 1414 | 1044 | 1229 | 810 | 1925 | 1367 | 2224 | 2969 | 2596 |
| L.S.D. between heights within seedings 5% | | 250 | 199 | 155 | 228 | N.S. | 191 | 256 | 238 | 188 |
| L.S.D. between seedings within heights 5% | | 354 | 203 | 198 | 222 | N.S. | 135 | 338 | 280 | 219 |
| Average — All three seedings | 1½″ | 1909 | 1780 | 1845 | — | — | — | 3008 | 3019 | 3014 |
| | 3″ | 1410 | 1279 | 1345 | — | — | — | 2248 | 2521 | 2384 |
| L.S.D. 5% | | 144 | 115 | 90 | — | — | — | 148 | 137 | 108 |
| Average — Two mixtures | 1½″ | 1769 | 1457 | 1613 | 1648 | 1858 | 1753 | 3417 | 3315 | 3366 |
| | 3″ | 1369 | 1056 | 1212 | 1255 | 1864 | 1560 | 2624 | 2920 | 2772 |
| L.S.D. 5% | | 154 | 144 | 105 | 161 | N.S. | 135 | 132 | 150 | 114 |

1,000 pounds of 4-16-12 and 25 pounds of borax per acre was made before seeding.

A split-plot design with four replications was used. The seeding mixtures were assigned to the whole plots and the cutting treatments to the split plots. Individual plots were 6 x 20 feet in size.

Oregon Certified Ladino clover, *Trifolium repens* L., was seeded alone and in mixture with either Climax timothy, *Phleum pratense* L., or Canadian smooth bromegrass, *Bromus inermis* Leyss. The rates of seeding per acre were 2 pounds for Ladino clover, 8 pounds for timothy and 16 pounds for smooth bromegrass. The clover seed was inoculated immediately prior to seeding. The companion crop was Vanguard oats sown at the rate of 1½ bushels per acre with a grain drill after the soil had been cultipacked. Following the sowing of the companion crop, the forage seeds were broadcast by hand and the plots were raked lightly for seed coverage. All seeding operations took place on June 12, 1954. The oats were cut when heading out on July 29 and removed from the plots. The regrowth of the companion crop was clipped on November 16 and removed from the experimental area. All the plots had a dense stand and were in excellent condition in the fall.

Forage yields were obtained in 1955 and 1956. Two heights of cutting, 1½ and 3 inches, and three frequencies of cutting, two, four and six times per season, were applied to each seeding. No cutting was made after September 10. One swath was taken through the entire length of each plot. Immediately after cutting, a representative sample was collected from each plot and hand separated into each of the seeded components (clover and grass fractions) and into miscellaneous plants (weeds and volunteering useful species). Each forage fraction was first air-dried and then oven-dried to constant weight.

## RESULTS AND DISCUSSION

### *Yields of Forage*

The average yields of dry matter in pounds per acre of the clover fraction, grass fraction and total production for each seeding at each cutting treatment and for each year are presented in Tables 1, 2 and 3.

Growing Ladino clover with a grass increased considerably the total production of dry matter per acre during both years. The largest yield of clover, however, was harvested when Ladino clover was grown alone, having no competition to sustain from the grass partner. The two mixtures did not differ significantly in the amount of clover produced. Of the three seedings, the Ladino clover-timothy association gave the highest yields of total forage over the 2-year period.

Bromegrass, because of its slow establishment, produced a rather small amount of grass the first harvest year in comparison with timothy. The following year, however, it yielded slightly more than the latter. The large increase in the bromegrass fraction from 1955 to 1956 was accompanied by a marked reduction in the clover fraction.

A significant response to height of cutting was obtained from all seedings in 1955 and 1956. Raising the height of cutting from 1½ to 3

TABLE 3. — EFFECT OF FREQUENCY OF CUTTING ON THE FRACTION AND TOTAL YIELDS OF DRY MATTER IN POUNDS PER ACRE FOR EACH SEEDING AND EACH HARVEST YEAR

| Seeding | Frequency of cutting | Clover fraction | | | Grass fraction | | | Total production | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | 1955 | 1956 | Average | 1955 | 1956 | Average | 1955 | 1956 | Average |
| Ladino clover alone | 2 | 2028 | 2794 | 2411 | — | — | — | 2028 | 2794 | 2411 |
| | 4 | 1948 | 2002 | 1975 | — | — | — | 1948 | 2002 | 1975 |
| | 6 | 1548 | 1433 | 1490 | — | — | — | 1548 | 1433 | 1490 |
| Ladino clover — timothy | 2 | 1334 | 1388 | 1361 | 2928 | 3015 | 2972 | 4262 | 4403 | 4333 |
| | 4 | 1747 | 1726 | 1736 | 1568 | 1310 | 1439 | 3315 | 3036 | 3175 |
| | 6 | 1388 | 910 | 1149 | 1334 | 1022 | 1178 | 2722 | 1932 | 2327 |
| Ladino clover — bromegrass | 2 | 1608 | 1144 | 1376 | 1500 | 3433 | 2466 | 3108 | 4577 | 3842 |
| | 4 | 1842 | 1433 | 1638 | 736 | 1380 | 1058 | 2578 | 2813 | 2696 |
| | 6 | 1495 | 938 | 1216 | 644 | 1006 | 825 | 2139 | 1944 | 2041 |
| L.S.D. between frequencies within seedings 5% | | 306 | 244 | 190 | 280 | 261 | 234 | 313 | 291 | 230 |
| L.S.D. between seedings within frequencies 5% | | 434 | 248 | 243 | 272 | 308 | 165 | 414 | 344 | 268 |
| Average — All three seedings | 2 | 1657 | 1775 | 1716 | — | — | — | 3132 | 3925 | 3528 |
| | 4 | 1846 | 1720 | 1783 | — | — | — | 2614 | 2617 | 2616 |
| | 6 | 1477 | 1094 | 1285 | — | — | — | 2136 | 1769 | 1952 |
| L.S.D. 5% | | 177 | 141 | 110 | — | — | — | 181 | 168 | 133 |
| Average — Two mixtures | 2 | 1471 | 1266 | 1368 | 2214 | 3224 | 2719 | 3685 | 4490 | 4087 |
| | 4 | 1794 | 1579 | 1687 | 1152 | 1345 | 1248 | 2946 | 2924 | 2935 |
| | 6 | 1442 | 924 | 1183 | 989 | 1014 | 1001 | 2431 | 1938 | 2184 |
| L.S.D. 5% | | 189 | 177 | 128 | 198 | 184 | 165 | 162 | 184 | 140 |

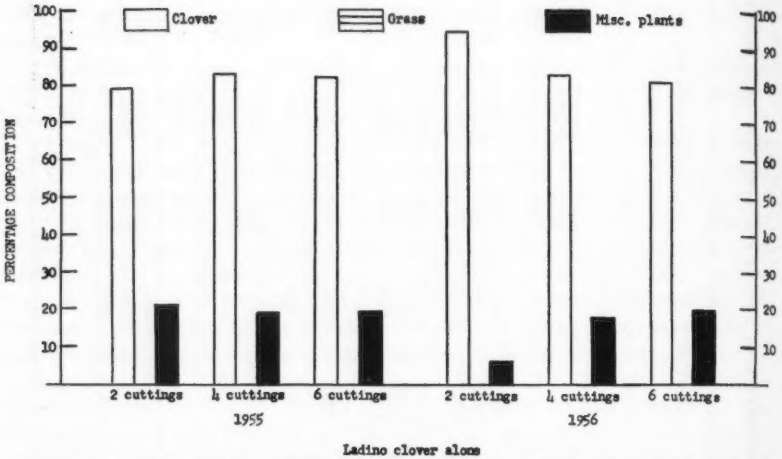


FIGURE 5. Effect of frequency of cutting on the botanical composition of forage harvested in 1955 and 1956 from Ladino clover grown alone.

inches depressed the fraction and total yields of dry matter of the three seedings each year, except in the grass fraction in the second harvest year where the yields were of the same magnitude under the two heights of cutting.

The number of times the forage was cut during the growing season caused a marked effect on the fraction and total yields of all seedings. The two mixtures exhibited a similar response to cutting frequency. Cutting four times per season resulted each year in more clover, but in much less grass and total production than cutting twice. The lowest fraction and total yields of dry matter were recorded under the system of six cuttings. Frequent defoliations markedly reduced the grass yields and total production. Both were depressed as the number of cuttings increased from two to four to six per season.

Ladino clover when seeded alone behaved differently to cutting frequency than when seeded in mixtures. The clover production varied inversely with the number of times the forage was cut during the growing season. The highest production of dry matter was recorded under the least frequent system of cutting.

The over-all response of the two mixtures for the 2-year period showed that plots cut four times to a height of 1½ inches gave the highest yields of clover, whereas those cut six times to a height of 3 inches produced the least clover. The highest and lowest yields of grass were obtained from plots cut twice to a height of 1½ inches and six times to a height of 3 inches, respectively. Ladino clover grown alone gave the highest and lowest yields of clover when cut twice to a height of 1½ inches and six times to a height of 3 inches, respectively.

The fraction and total yields of forage harvested per acre differed between years. Ladino clover produced more clover the second harvest

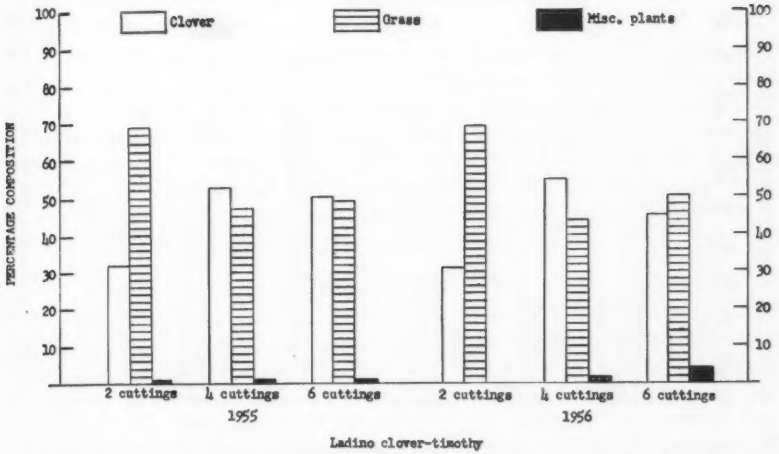


FIGURE 6. Effect of frequency of cutting on the botanical composition of forage harvested in 1955 and 1956 from a Ladino clover-timothy mixture.

year when grown alone, and the first harvest year when associated with a grass. Timothy yielded more in 1955 than in 1956 while bromegrass was far more productive in 1956 than in 1955. In total production, the Ladino clover-timothy association yielded more the first than the second harvest year while the reverse was true for the other two seedings.

*Botanical Composition of Forage*

The botanical composition of the forage harvested in 1955 and 1956 was based on the yields of dry matter of the clover fraction, grass fraction and miscellaneous plants. The effects of seedings and cutting treatments on the botanical composition of forage are illustrated in Figures 1 to 7.

Pure seedings of Ladino clover, irrespective of cutting treatments, contained much more clover than the two clover-grass associations during each year. Likewise, miscellaneous plants were more prevalent in Ladino clover grown alone largely as a result of invading volunteering grasses. The percentages of clover and grass in the clover-timothy mixture did not vary to any extent from one year to the next while they changed materially in the clover-bromegrass mixture. In the latter case, the clover decreased by 22.7 per cent and the grass increased by a corresponding amount in 1956 in comparison with 1955. The amount of miscellaneous plants was negligible in the two clover-grass associations.

Height of cutting exerted little if any influence on the clover content of the forage harvested from the mixed swards in 1955. However, in 1956, cutting to 3 inches lowered the clover fraction by 8.4 per cent and 4.7 per cent in the timothy and bromegrass mixtures, respectively, as compared with the 1½-inch cutting. Conversely the grasses increased by raising the height of cutting. Ladino clover seeded alone had 4.6 per cent more clover when cut to a height of 1½ inches than when cut to a height

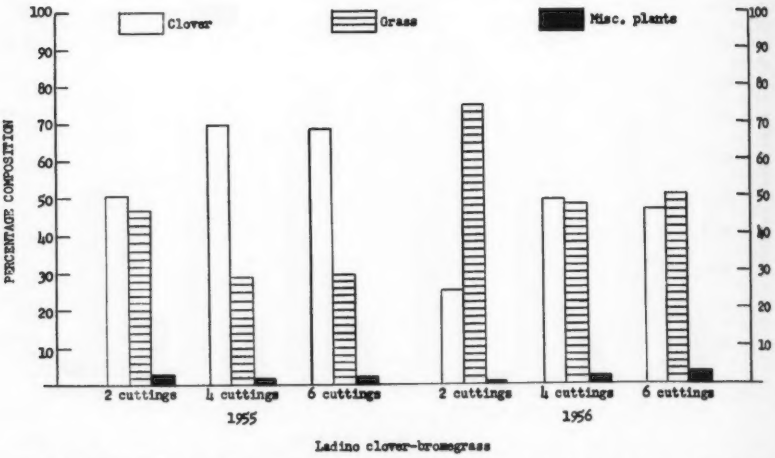


FIGURE 7. Effect of frequency of cutting on the botanical composition of forage harvested in 1955 and 1956 from a Ladino clover-bromegrass mixture.

of 3 inches in 1955. The following year, the percentages of clover averaged the same for the two heights of cutting but at a higher level than in 1955.

Frequency of cutting caused a distinct effect on the clover-grass-miscellaneous plants ratio of forage harvested. Ladino clover grown alone had slightly less clover in 1955 when cut twice than when cut either four or six times. In 1956, the infrequently cut plots contained the most clover, an increase of 13.8 per cent over 1955, and the least miscellaneous plants. Cutting four or six times resulted in similar amounts of clover and miscellaneous plants in both years.

The Ladino clover-timothy mixture contained the least clover and the most grass during both years under the system of two cuttings per season. Harvested for hay (two cuttings), this mixture had more than twice as much grass as clover. Ladino clover was most prevalent during each year under the system of four cuttings and in a higher proportion than the grass.

The Ladino clover-bromegrass mixture also showed the lowest and the highest percentages of clover when cut twice and four times, respectively, during both 1955 and 1956. Forage cut six times was almost equal to that cut four times in its clover content. Under all frequencies of cutting, the percentage of clover declined markedly from the first to the second harvest year while the percentage of bromegrass increased in a like manner. The forage harvested at the hay stage (two cuttings) contained a much higher percentage of grass than the forage cut at immature stages of growth (four and six cuttings).

With the two mixtures, cutting four times per season to a height of 1½ inches contributed to maintaining the proportion of clover at the highest level during each year, whereas the most lenient system of cutting,

i.e. two cuttings to a height of 3 inches, resulted in the lowest amount of clover the second harvest year.

The results of these investigations have shown the good adaptation of Ladino clover to the Eastern Townships of Quebec. Grown in association with a compatible grass, it will provide a high tonnage of forage of excellent quality. Grazing in rotation rather intensively for short periods, as suggested by the management system involving four cuttings to a height of 1½ inches, would ensure a high return and maximum duration. A larger production can be expected from the grass associate when the mixture is harvested for hay but at the expense of the clover stand.

## ACKNOWLEDGEMENTS

Deep appreciation is expressed to Dale Smith and J. H. Torrie, of the University of Wisconsin, for their very helpful suggestions and guidance in these studies; and to A. E. Ouellette and L. Lachance, Research Officers at the Experimental Farm, Lennoxville, Que., for assistance in carrying out the field work. A scholarship granted by the Quebec Agricultural Research Council to the author during his studies at the University of Wisconsin is gratefully acknowledged.

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# EFFECTS OF CUTTING TREATMENTS ON LADINO CLOVER GROWN ALONE AND IN MIXTURE WITH GRASSES

## II. CHEMICAL COMPOSITION OF FORAGE[1]

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### ABSTRACT

Ladino clover grown alone and in mixture with timothy and smooth bromegrass was cut two, four and six times per season to a height of 1½ and 3 inches. Forage harvested in 1955 and 1956 from a seeding made in 1954 at the Experimental Farm, Lennoxville, Quebec, was chemically analysed.

Ladino clover contained more K but less N-free extract and Ca when grown alone than when associated with a grass. Bromegrass had a higher content of crude protein, ash, P and K, and a lower content of N-free extract than timothy.

Height of cutting failed to modify the chemical composition of the grasses. In the clover, close cutting reduced the fat and K contents and increased the ash content.

The crude protein content increased and the crude fibre and N-free extract contents decreased in both forage fractions with increasing cutting frequencies. Fat was highest in the clover cut four times and in the grasses cut four and six times. The ash content in the clover was not significantly altered by cutting frequency but tended to decrease with increasing maturity in the grasses. The Ca content was lowest in the clover cut six times and in the grasses cut twice. In both forage fractions, the P and K contents were higher with either four or six cuttings than with two cuttings.

### INTRODUCTION

The effects of cutting treatments on the productivity of Ladino clover grown alone and in mixture with timothy and bromegrass and on the botanical composition of the forage harvested from these seedings were reported in a previous paper (2). The present report deals with the effects of the same managerial treatments on the chemical composition of the clover and grasses. The study was conducted during 1955 and 1956 at the Experimental Farm, Lennoxville, Quebec.

### REVIEW OF LITERATURE

Ladino clover has long been recognized as a very nutritious forage. It contains high percentages of the desirable feed components according to Plummer (5), and is high in nitrogen and mineral elements as shown by Stewart and Bear (6). Wedin *et al.* (7) found that the average contents of nitrogen, phosphorus and potassium decreased in Ladino clover as the growth progressed from pasture to intermediate to hay stage. Peterson and Hagan (3) reported that the crude protein diminished and the crude fibre increased as the forage became older; the N-free extract showed no definite trend while the ether-extract and ash contents tended to decrease as the interval between cuttings was increased. Results obtained by Brown and Munsell (1) indicated that close cutting was conducive to lower protein content and higher fibre content than high cutting.

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[1]Part of a thesis submitted in partial fulfilment of the requirements of the degree of Doctor of Philosophy, University of Wisconsin. 1958.

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TABLE 1. — EFFECT OF SEEDINGS ON THE AVERAGE CHEMICAL COMPOSITION OF THE CLOVER AND GRASS FRACTIONS OF FORAGE HARVESTED IN 1955 AND 1956

| Seeding | Fraction | Percentage | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Crude protein | Crude fibre | N-free extract | Fat | Ash | Ca | P | K |
| Ladino clover alone | Clover | 26.10 | 18.27 | 44.71 | 1.79 | 9.13 | 1.74 | 0.32 | 1.80 |
| Ladino clover — timothy | Clover | 25.55 | 18.16 | 45.80 | 1.78 | 8.71 | 1.89 | 0.31 | 1.59 |
| Ladino clover — bromegrass | Clover | 25.98 | 17.84 | 45.58 | 1.83 | 8.77 | 1.81 | 0.31 | 1.65 |
| L.S.D. 5% | | N.S. | N.S. | 0.85 | N.S. | N.S. | 0.08 | N.S. | 0.08 |
| Ladino clover — timothy | Grass | 15.81 | 27.66 | 46.35 | 2.79 | 7.39 | 0.58 | 0.29 | 2.22 |
| Ladino clover — bromegrass | Grass | 19.35 | 27.28 | 41.90 | 2.74 | 8.73 | 0.61 | 0.33 | 2.59 |
| L.S.D. 5% | | 1.69 | N.S. | 1.89 | N.S. | 0.45 | N.S. | 0.04 | 0.15 |

Phillips *et al.* (4), reporting on the chemical composition of eight grasses, including timothy and bromegrass, at various stages of growth, have shown that the percentages of protein, ether extract and soluble ash decreased, and those of crude fibre and N-free extract increased as the plants advanced in maturity.

## MATERIALS AND METHODS

This study involved three forage seedings, viz., Oregon Certified Ladino clover seeded alone and in mixture with either Climax timothy or Canadian smooth bromegrass. Each seeding was cut two, four and six times per season to a height of 1½ and 3 inches above the ground. A split-plot design with four replications was used. Further details on general technique were reported elsewhere (2).

Data were obtained in 1955 and 1956 from a seeding made in 1954. A representative sample was collected from each plot immediately after cutting and hand separated into each of the seeded components (clover and grass fractions) and into miscellaneous plants (weeds and volunteering useful species). The fraction samples were first air-dried and then oven-dried to constant weight. Chemical analysis was made on the bulked samples of the grass fraction and of the clover fraction from the four replications of each treatment.

In the combined statistical analysis for the 2 years, the interactions with years were pooled to provide an estimate of error since bulked samples of each forage fraction were used for chemical analyses. As the statistical analysis indicated no evidence of interaction involving seedings, least significant differences were not computed to provide comparisons between managements such as associations within heights, etc.

## RESULTS AND DISCUSSION

The average chemical composition of the clover and grass fractions for the 2 years as influenced by the main factors is given in Tables 1, 2 and 3. Only the average composition is presented in order to conserve space.

The chemical composition of the clover fraction was very similar for the three seedings over the 2-year period. The average contents of crude protein, crude fibre, fat, ash and phosphorus did not differ significantly. Ladino clover, however, contained less N-free extract and calcium but more potassium when grown alone than when associated with a grass.

Timothy and bromegrass differed markedly in their chemical composition. Timothy contained significantly less crude protein, ash, phosphorus and potassium but more N-free extract than bromegrass. The percentages of crude fibre, fat and calcium were similar for both grasses.

There is a large body of evidence indicating that legumes, in general, are much higher in crude protein and calcium and lower in crude fibre than grasses. Results obtained in this study are no exception. The harvested portion of the Ladino clover plants was composed almost entirely of leaves. Leaves contain more crude protein and less crude fibre than stems. Therefore, Ladino clover had a high content of crude protein and a low content of crude fibre as compared with the grasses, the herbage of

TABLE 2.—EFFECT OF HEIGHT OF CUTTING ON THE AVERAGE CHEMICAL COMPOSITION OF THE CLOVER AND GRASS FRACTIONS OF FORAGE HARVESTED IN 1955 AND 1956 FOR EACH SEEDING

| Seeding | Fraction | Height of cutting | Percentage | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Crude protein | Crude fibre | N-free extract | Fat | Ash | Ca | P | K |
| Ladino clover alone | Clover | 1½″ | 25.78 | 18.62 | 44.36 | 1.77 | 9.46 | 1.76 | 0.32 | 1.78 |
| | | 3″ | 26.42 | 17.92 | 45.07 | 1.80 | 8.79 | 1.72 | 0.31 | 1.83 |
| Ladino clover — timothy | Clover | 1½″ | 25.02 | 18.57 | 45.77 | 1.75 | 8.89 | 1.89 | 0.32 | 1.54 |
| | | 3″ | 26.07 | 17.75 | 45.84 | 1.81 | 8.53 | 1.89 | 0.30 | 1.63 |
| Ladino clover — bromegrass | Clover | 1½″ | 25.89 | 17.96 | 45.55 | 1.77 | 8.83 | 1.84 | 0.31 | 1.61 |
| | | 3″ | 26.07 | 17.72 | 45.60 | 1.90 | 8.71 | 1.78 | 0.31 | 1.69 |
| Average | | 1½″ | 25.56 | 18.38 | 45.23 | 1.77 | 9.06 | 1.83 | 0.32 | 1.64 |
| | | 3″ | 26.18 | 17.80 | 45.50 | 1.84 | 8.68 | 1.80 | 0.31 | 1.72 |
| L.S.D. 5% | | | N.S. | N.S. | N.S. | 0.06 | 0.32 | N.S. | N.S. | 0.08 |
| Ladino clover — timothy | Grass | 1½″ | 15.76 | 27.27 | 46.69 | 2.74 | 7.54 | 0.58 | 0.30 | 2.25 |
| | | 3″ | 15.87 | 28.04 | 46.01 | 2.84 | 7.24 | 0.58 | 0.27 | 2.18 |
| Ladino clover — bromegrass | Grass | 1½″ | 19.40 | 27.11 | 41.92 | 2.71 | 8.86 | 0.62 | 0.33 | 2.63 |
| | | 3″ | 19.29 | 27.46 | 41.89 | 2.77 | 8.59 | 0.60 | 0.32 | 2.56 |
| Average | | 1½″ | 17.58 | 27.19 | 44.31 | 2.72 | 8.20 | 0.60 | 0.32 | 2.44 |
| | | 3″ | 17.58 | 27.75 | 43.95 | 2.81 | 7.91 | 0.59 | 0.30 | 2.37 |
| L.S.D. 5% | | | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. | N.S. |

TABLE 3. — EFFECT OF FREQUENCY OF CUTTING ON THE AVERAGE CHEMICAL COMPOSITION OF THE CLOVER AND GRASS FRACTIONS OF FORAGE HARVESTED IN 1955 AND 1956 FOR EACH SEEDING

| Seeding | Fraction | Frequency of cutting | Percentage | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Crude protein | Crude fibre | N-free extract | Fat | Ash | Ca | P | K |
| Ladino clover alone | Clover | 2 | 23.06 | 20.42 | 45.59 | 1.81 | 9.12 | 1.78 | 0.29 | 1.60 |
| | | 4 | 27.12 | 17.62 | 44.36 | 1.85 | 9.05 | 1.76 | 0.32 | 1.88 |
| | | 6 | 28.11 | 16.78 | 44.20 | 1.70 | 9.21 | 1.68 | 0.34 | 1.93 |
| Ladino clover — timothy | Clover | 2 | 21.82 | 20.77 | 46.83 | 1.71 | 8.87 | 1.97 | 0.28 | 1.47 |
| | | 4 | 26.73 | 17.24 | 45.41 | 1.87 | 8.75 | 1.91 | 0.32 | 1.65 |
| | | 6 | 28.09 | 16.46 | 45.17 | 1.76 | 8.52 | 1.79 | 0.33 | 1.65 |
| Ladino clover — bromegrass | Clover | 2 | 22.75 | 20.19 | 46.39 | 1.78 | 8.89 | 1.88 | 0.28 | 1.47 |
| | | 4 | 26.81 | 16.97 | 45.57 | 1.94 | 8.71 | 1.83 | 0.32 | 1.69 |
| | | 6 | 28.38 | 16.37 | 44.77 | 1.77 | 8.71 | 1.72 | 0.33 | 1.79 |
| Average | | 2 | 22.54 | 20.46 | 46.27 | 1.77 | 8.96 | 1.88 | 0.28 | 1.51 |
| | | 4 | 26.88 | 17.28 | 45.11 | 1.89 | 8.84 | 1.83 | 0.32 | 1.74 |
| | | 6 | 28.19 | 16.54 | 44.72 | 1.74 | 8.81 | 1.73 | 0.33 | 1.79 |
| L.S.D. 5% | | | 1.26 | 0.76 | 0.85 | 0.07 | N.S. | 0.08 | 0.02 | 0.09 |
| Ladino clover — timothy | Grass | 2 | 12.03 | 31.10 | 47.85 | 2.62 | 6.40 | 0.52 | 0.25 | 1.91 |
| | | 4 | 17.26 | 26.34 | 45.70 | 2.87 | 7.83 | 0.59 | 0.31 | 2.43 |
| | | 6 | 18.15 | 25.53 | 45.51 | 2.88 | 7.93 | 0.64 | 0.30 | 2.31 |
| Ladino clover — bromegrass | Grass | 2 | 14.98 | 30.31 | 44.17 | 2.66 | 7.88 | 0.55 | 0.27 | 2.23 |
| | | 4 | 20.15 | 26.46 | 41.64 | 2.77 | 8.98 | 0.60 | 0.35 | 2.75 |
| | | 6 | 22.92 | 25.07 | 39.90 | 2.79 | 9.32 | 0.68 | 0.36 | 2.80 |
| Average | | 2 | 13.50 | 30.71 | 46.01 | 2.64 | 7.14 | 0.54 | 0.26 | 2.07 |
| | | 4 | 18.70 | 26.40 | 43.67 | 2.82 | 8.41 | 0.59 | 0.33 | 2.59 |
| | | 6 | 20.54 | 25.30 | 42.70 | 2.84 | 8.62 | 0.66 | 0.33 | 2.55 |
| L.S.D. 5% | | | 2.07 | 0.73 | 2.31 | N.S. | 0.56 | 0.08 | 0.05 | 0.19 |

which was composed of both leaf and stem portions. The two grasses had a much higher percentage of fat than the clover. The calcium content in the clover was about three times higher than that in the grasses while the potassium content was less than in the grasses.

Height of cutting, in the main, had little effect on the chemical composition of species. It failed to establish a trend in the grass fraction and no significant difference was found for any one constituent. In the clover fraction, close cutting reduced the fat and potassium contents but increased the ash content. None of the other constituents was significantly different as a result of height of cutting.

Frequency of cutting or stage of growth exerted a marked effect on the chemical composition of both forage fractions. In the present investigations, the plants were harvested at three stages of growth: at a very young stage under the system of six cuttings per season; at a slightly more advanced but still immature stage, under the system of four cuttings; and at the hay stage when cut twice. Differences in the chemical composition, in general, were smaller between the plants cut six and four times – at immature stages of growth – than between those cut four times and twice – at immature and hay stages.

Results obtained with both the clover and grass fractions substantiate the well established fact that the younger the forage, the higher the crude protein content and the lower the crude fibre content. The percentage of crude protein decreased and that of crude fibre increased as the number of cuttings decreased from six to four to two per season, i.e., with advancing maturity. The N-free extract content in both the legume and grass fractions followed a trend similar to that of the crude fibre content, being at the highest level in forage infrequently cut. The percentage of fat was highest in the clover cut four times and in the grass species cut four and six times.

The ash content in the clover fraction was not significantly altered by frequency of cutting. In the grass fraction, it tended to decrease with increasing maturity. The percentage of calcium followed an opposite trend according to species. In the clover fraction, it tended to increase with advancing stage of growth while in the grass fraction the reverse was true. The phosphorus and potassium contents in the plant tissue of both forage fractions were significantly lower under the system of two cuttings than under either four or six cuttings.

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# THE ASSOCIATION OF FLOWERING HABIT WITH WINTER SURVIVAL IN RED AND ALSIKE CLOVER DURING THE SEEDLING YEAR OF GROWTH[1]

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[Received for publication September 9, 1959]


## ABSTRACT

The percentage of plants that flowered in the seedling year in spaced populations of red and alsike clovers at Madison and Arlington, Wisconsin, was highest in the earliest seedings (May 15) and decreased with later seeding dates. No plants flowered in the July 15th seedings. The percentage of winterkilling during the first winter was higher in the flowering plants of each clover than in the non-flowering plants. Differences in flowering and winter survival were noted among clover strains and in winter survival among plant types within strains.

Prevention of flowering in medium red clover by removing flower buds, flowering stems and/or elongating tillers resulted in greater vegetative vigour and winter survival. Plants that were allowed to flower freely in the seedling year in spaced populations had a smaller number of non-flowering crown tillers, a smaller width of crown, a lower dry weight of crown, root and total available carbohydrates in the roots, less root branching, a slightly lower percentage of total available carbohydrates in the roots, and more winterkilling during the first winter than plants that were prevented from flowering. These responses may help in part to explain the benefits attributed to clipping red clover in the seedling year.


## INTRODUCTION

The killing or weakening of stands during the winter period is one of the most important problems related to the culture of red and alsike clover in the northern areas. The growth habit and flowering of the plants during the seedling year, however, appear to have a considerable influence on survival during the first winter. Thus, work was initiated to study the influence of the date of seeding red and alsike clover on flowering and winter survival during the seedling year, and to study the influence of treatments that prevented flowering during the seedling year on the growth and winter survival of medium red clover.

## LITERATURE REVIEW

Gorman (5), Keller and Peterson (6), and Ludwig *et al.* (8) indicate that medium red clover requires a photoperiod of at least 13 to 14 hours to flower. Longer photoperiods are needed for flowering in mammoth red clover (5, 6). Keller and Peterson (6) found that Emerson medium red clover and mammoth red clover produced no flower heads under 10-hour days but both strains produced flowers under 18-hour days. With 14-hour days, the medium red clover produced numerous flowers while the mammoth produced almost none. Gorman (5) and Ludwig *et al.* (8) found that flowering in medium and mammoth red clover occurred earlier and more profusely as the photoperiod was lengthened. Ludwig *et al.* (8) also noted that plants within a variety responded differentially to daylength. Bird (2) distinguished five growth types in seedling-year populations of red

[1]Contribution from the Department of Agronomy, Wisconsin Agricultural Experiment Station, Madison, Wis. Published with approval of the Director, Wisconsin Agricultural Experiment Station. Part of a thesis submitted by the senior author to the University of Wisconsin in partial fulfilment of requirements for the Ph.D. degree. 1959.

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clover transplanted to the field in early summer. Two were non-flowering types and three were flowering types. Smith (9) found that date of seeding had a marked influence on the proportion of flowering and non-flowering plant types in medium red clover. In spaced populations of Wisconsin Common medium red clover, almost all plants seeded in mid-May produced flowering stems by early October of the seeding year. The percentage of flowering plants declined steadily with later seedings until virtually no plants flowered in mid-July seedings. Only the plants which were well-established when exposed to long day lengths of mid-summer apparently manifested their flowering response. Thus, date of seeding and rapidity of plant development can influence the amount of flowering in the seedling year. Bird (2), Emery (4), and Smith (9) have shown an association between habit of growth in the seedling year and winter survival in red clover. Non-flowering plants survived the first winter better than flowering plants.

## MATERIALS AND METHODS

### *Experiment I*

Wisconsin Common, Lakeland, and Dollard medium red clover (*Trifolium pratense* L.), Altaswede mammoth red clover (*Trifolium pratense* L.), and Wisconsin Common alsike clover (*Trifolium hybridum* L.) were established at Madison and Arlington, Wisconsin, in 1957 and 1958, respectively.

Spaced populations of the clovers were established as described by Smith (9). Seedings were made each year at five different dates: May 15, June 1, June 15, July 1, and July 15. A randomized complete block design with six replications was used. The seed was sown in rows 2 feet apart. Approximately 4 weeks after seeding, the plants were thinned to one plant every 18 inches in the row. The average population of each clover for each seeding date was about 150 plants each year. The plants were classified in early October of each seeding year on the basis of growth habit and flowering into five types in the same manner as described by Bird (2). The five plant types were: *type 1*, plants that produced only a rosette of leaves and unelongated tillers; *type 2*, plants that produced mostly a leafy rosette but had one or two elongated non-flowering tillers or stems; *type 3*, plants that produced a prominent rosette but with several flowering stems; *type 4*, plants that produced a weak rosette with many flowering stems generally upright; and *type 5*, plants that produced virtually no rosette with many upright flowering stems. Diseased plants were removed from the populations so that survival over winter was based on healthy and vigorous plants. The top growth of all plants was removed in late November so that differentials in snow cover would not occur during winter. The percentages of winterkilling were ascertained in the spring after growth commenced by recording the number of plants dead and the number alive.

### *Experiment II*

Spaced populations of Wisconsin Common medium red clover were established at Madison in 1956 and 1957, and at Arlington in 1958. Dollard

medium red clover was used also in 1958. Each year the seedings were made on May 15 ±1 day. The seeding and establishment procedures were the same as in Experiment I. Each year, several management treatments were applied to the seedling plants in a randomized complete block design. The number of replicates were five in 1956, ten in 1957, and six in 1958. Each plot consisted of two adjacent rows in 1956 and a single row in 1957 and 1958. The number of plants per row averaged from 36 to 40 plants each year.

Plants in treatment 1 (T1), the check treatment, were allowed to grow and flower freely throughout the entire growing season. All inflorescences in the bud stage were pulled from the stems in T2 at weekly intervals from early to mid-July until early October of each year. All tillers in T3 were cut off with a sharp knife close to the crown as they began to elongate into stems. This treatment was initiated in early- to mid-July and repeated at weekly intervals until August 31. Plants in T4 were allowed to grow and flower freely until August 31. On this date, elongating tillers and flowering stems were removed close to the crown leaving only the rosette of unelongated tillers and leaves. T5 was the same as T4, except that elongating tillers and flowering stems were removed on July 31 and again on August 31. T5 was not used in 1956. The plants were classified in early October of each seeding year on the basis of growth habit and flowering into five types as described in Experiment I. Similarly, the top growth of all plants was removed in late November and the percentages of winterkilling were ascertained during the following spring.

The plants in one replicate in 1957 and in one replicate in 1958 were used for laboratory studies. Twenty-five plants were dug from each treatment in late October of each of the 2 seeding years. Each plant was separated into root and crown. The crown included the leaves and all non-flowering tillers. Flowering stems, when present, were cut close to the crown and discarded. The width of crown was then measured in inches in two different directions. The average of the two measurements and the number of tillers on each crown were recorded. The roots were cut to 5 inches in length and washed. The root and crown of each plant were placed in individual bags and dried at 140°F. The dry weights were recorded. After drying, each root was classified on the basis of abundance of branching into three groups: *1* – not branched or essentially tap-rooted; *2* – moderately branched, and *3* – abundantly branched. Roots from each treatment were bulked and analysed for the percentage of total available carbohydrates as described previously (9). Carbohydrates were calculated as glucose on a dry weight basis at 70°C.

## RESULTS

### *Influence of Date of Seeding on Flowering and Winter Survival*

The average percentages of each of five plant types in October of the seeding year in the spaced populations of red and alsike clover as influenced by five different seeding dates are shown in Table 1. The highest percentage of flowering plants (plant types 3, 4, and 5) in each clover occurred at the earliest seeding date, May 15. Virtually all plants of the three

TABLE 1. — THE AVERAGE PERCENTAGES OF EACH OF FIVE PLANT TYPES IN OCTOBER OF THE SEEDLING YEAR IN SPACED POPULATIONS OF SEVERAL CLOVER STRAINS AS INFLUENCED BY THE DATE OF SEEDING DURING TWO DIFFERENT YEARS 1957 AND 1958, AND THE AVERAGE PERCENTAGES OF WINTERKILLING DURING ONE WINTER, 1957-58

| Clover strains and plant types | Percentages of plants for seeding dates | | | | | % Killing winter of 1957-58[1] |
|---|---|---|---|---|---|---|
| | May 15 | June 1 | June 15 | July 1 | July 15 | |
| DOLLARD | | | | | | |
| 1 | 0.5 | 4.3 | 36.8 | 89.6 | 99.4 | 15.5 |
| 2 | 1.8 | 5.8 | 14.8 | 8.0 | 0.6 | 14.3 |
| 3 | 10.5 | 23.7 | 20.8 | 2.1 | 0 | 28.2 |
| 4 | 41.4 | 34.1 | 20.0 | 0 | 0 | 50.5 |
| 5 | 45.8 | 32.1 | 7.6 | 0.3 | 0 | 77.5 |
| % Winterkilling 1957-58 | 68.0 | 56.0 | 30.0 | 10.0 | 19.0 | |
| LAKELAND | | | | | | |
| 1 | 0.4 | 1.2 | 27.6 | 85.7 | 100.0 | 21.1 |
| 2 | 0.4 | 1.8 | 10.8 | 8.2 | 0 | 37.5[2] |
| 3 | 10.9 | 15.0 | 27.2 | 1.5 | 0 | 35.3 |
| 4 | 44.5 | 35.8 | 21.3 | 1.0 | 0 | 56.7 |
| 5 | 43.8 | 46.2 | 13.1 | 3.6 | 0 | 79.4 |
| % Winterkilling 1957-58 | 65.0 | 64.0 | 34.0 | 22.0 | 26.0 | |
| WISCONSIN COMMON | | | | | | |
| 1 | 0.7 | 1.5 | 17.8 | 66.4 | 97.2 | 31.0 |
| 2 | 0.5 | 1.5 | 12.9 | 20.1 | 2.8 | 45.5 |
| 3 | 5.3 | 5.8 | 13.9 | 0.6 | 0 | 22.7[2] |
| 4 | 37.6 | 31.1 | 29.9 | 6.7 | 0 | 48.3 |
| 5 | 55.9 | 60.1 | 26.5 | 6.2 | 0 | 79.7 |
| % Winterkilling 1957-58 | 68.0 | 71.0 | 45.0 | 30.0 | 24.0 | |
| ALTASWEDE | | | | | | |
| 1 | 83.0 | 95.0 | 100.0 | 100.0 | 100.0 | 20.7 |
| 2 | 6.1 | 3.7 | 0 | 0 | 0 | –[2] |
| 3 | 9.3 | 1.3 | 0 | 0 | 0 | –[2] |
| 4 | 1.0 | 0 | 0 | 0 | 0 | –[2] |
| 5 | 0.6 | 0 | 0 | 0 | 0 | –[2] |
| % Winterkilling 1957-58 | 47.0 | 18.0 | 16.0 | 17.0 | 22.0 | |
| ALSIKE | | | | | | |
| 1 | 0 | 2.5 | 44.1 | 95.4 | 100.0 | 16.7 |
| 2 | 0 | 0 | 1.3 | 2.6 | 0 | –[2] |
| 3 | 37.5 | 48.4 | 35.6 | 2.0 | 0 | 20.0 |
| 4 | 42.2 | 33.7 | 14.7 | 0 | 0 | 48.2 |
| 5 | 20.3 | 15.4 | 4.3 | 0 | 0 | 66.1 |
| % Winterkilling 1957-58 | 52.0 | 40.0 | 22.0 | 18.0 | 17.0 | |

[1]The 1958 seedings were almost completely killed during the winter of 1958-59.
[2]Plant numbers too small for reliable results.

TABLE 2. — THE AVERAGE PERCENTAGES OF KILLING DURING THE WINTER OF 1957-58 IN FIVE PLANT TYPES IN SPACED POPULATIONS OF SEVERAL CLOVERS SEEDED ON JUNE 15 IN 1957[1]

| Clover strains | Per cent winterkilling in each plant type | | | | | Average |
|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | |
| Dollard | 16.7 | 21.0 | 29.3 | 39.6 | 55.0 | 30.0 |
| Lakeland | 20.5 | 23.1 | 27.7 | 42.0 | 63.6 | 34.0 |
| Wisconsin Common | 29.4 | 33.3 | 33.3 | 46.3 | 65.0 | 45.0 |
| Average | 22.2 | 25.8 | 30.1 | 42.6 | 61.2 | |
| Altaswede | 16.0 | –[1] | –[1] | –[1] | –[1] | 16.0 |
| Alsike | 19.0 | –[1] | 12.7 | 30.0 | –[1] | 20.0 |

[1]Plant numbers too small for reliable results

TABLE 3. — THE AVERAGE PERCENTAGES OF WINTERKILLING DURING THE WINTER OF 1957-58 OVER ALL PLANT TYPES AND DATES OF SEEDING

| Clover strains | No. of plants | % Winterkilling |
|---|---|---|
| Wisconsin Common | 739 | 48.7 |
| Lakeland | 833 | 41.3 |
| Dollard | 837 | 35.8 |
| Alsike | 783 | 28.4 |
| Altaswede | 676 | 22.5 |

medium red clover varieties and of alsike seeded on May 15 flowered during the seedling year. Only a small percentage of the Altaswede mammoth red clover plants flowered. The percentage of flowering plants declined steadily from the early to the later seeding dates until no plants flowered in the July 15th seedings. The greatest diversity of plant types within Altaswede occurred in the mid-May seeding and in the other clovers in the mid-June seedings.

Among the three medium red clover varieties it was noted that the percentage of flowering plants (plant types 3, 4, and 5) at each seeding date was lowest in Dollard and highest in Wisconsin Common, while Lakeland was intermediate.

Differentials in winterkilling among plant types, dates of seeding, and clover strains were very apparent following the winter of 1957-58. Virtually all of the plants were killed during the winter of 1958-59. Therefore, the winterkilling data are limited to the 1957-58 results

TABLE 4. — THE AVERAGE PERCENTAGES OF PLANTS OF EACH OF FIVE PLANT TYPES IN SPACED POPULATIONS OF WISCONSIN COMMON MEDIUM RED CLOVER IN OCTOBER OF THE SEEDLING YEARS OF 1956, 1957, AND 1958 AS INFLUENCED BY MANAGERIAL TREATMENTS AND THE PERCENTAGES OF WINTER SURVIVAL DURING THE FIRST WINTER

| Treatments | Per cent of plants in types | | | | | % Winter survival[1] | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 1956-57 | 1957-58 |
| 1 | 0 | 0 | 28 | 40 | 32 | 0.7 | 42 |
| 2 | 0 | 0 | 74 | 22 | 4 | 11.2[3] | 47 |
| 3 | 17 | 27 | 38 | 14 | 4 | 12.2[3] | 74[3] |
| 4 | 23 | 25 | 26 | 22 | 4 | 4.3 | 63[3] |
| 5[2] | 6 | 30 | 28 | 30 | 6 | — | 73[3] |

[1]1958 populations were almost completely killed during the winter of 1958-59.
[2]Treatment applied only during 1957 and 1958.
[3]Significantly better survival than treatment 1 at 1% level.

(Table 1). Winterkilling over all dates of seeding was found generally to be least in plant type 1 and highest in type 5 in each of the clovers. Winterkilling of the plant types, however, was confounded with the influence of seeding date since when the data were averaged over all seeding dates, flowering plants were on the average older than non-flowering plants. The amount of winterkilling in the five plant types probably is best obtained from the June 15th seedings where the greatest diversity of plant types of the same age occurred in all but Altaswede (Table 2). Winterkilling on this basis was again least in plant type 1 and increased progressively to type 5, and this is most clearly shown in the three medium red clover varieties. Among the medium red clover varieties, winterkilling in each plant type generally was greatest in Wisconsin Common and least in Dollard, while Lakeland was intermediate.

The percentage of winterkilling over all plant types was in general highest in the early seeding dates and lowest in the later seeding dates (Table 1). The higher amount of killing in the early seeding dates was probably a reflection of the higher percentage of flowering plants in these populations.

The percentages of winterkilling for each clover over all plant types and seeding dates are shown in Table 3. Altaswede was winterkilled the least, followed in order by Alsike, Dollard, Lakeland, and Wisconsin Common.

*Influence of Management on the Development and Winter Survival of Medium Red Clover Plants during the Seedling Year*

The influence of treatments that allowed or prevented flowering during the seedling year on the growth and winter survival of Wisconsin Common medium red clover is shown in Tables 4 and 5.

TABLE 5. — THE 2-YEAR AVERAGE OF VARIOUS CHARACTERISTICS OF THE CROWNS AND ROOTS OF WISCONSIN COMMON MEDIUM RED CLOVER AS INFLUENCED BY FIVE DIFFERENT MANAGERIAL TREATMENTS APPLIED DURING THE SEEDLING YEARS OF 1957 AND 1958[1]

| Treatments | Non-flowering tillers[2] | Width of spread of crown | Dry weight of non-flowering tillers and crown | Type of root | Dry weight of root | Total available carbohydrates in root | Weight of carbohydrates[4] |
|---|---|---|---|---|---|---|---|
| | No. | Inches | Gm. | Index[3] | Gm. | % | Gm. |
| 1 (untreated) | 8 c | 8.2 c | 5.9 c | 1.46 b | 4.1 b | 37.0 | 1.5 |
| 2 | 12 b | 10.6 b | 12.1 b | 2.26 a | 7.7 a | 39.5 | 3.0 |
| 3 | 16 a | 15.1 a | 24.0 a | 2.24 a | 7.3 a | 41.2 | 3.0 |
| 4 | 13 ab | 11.3 b | 12.5 b | 1.96 a | 5.1 b | 40.5 | 2.1 |
| 5 | 16 a | 12.4 b | 16.7 b | 1.96 a | 5.8 ab | 39.7 | 2.3 |
| C.V. | 10% | 6.3% | 12.5% | 7.5% | 11.7% | — | — |

[1]Average per plant.
[2]Any two means followed by same letter are not significantly different at 5% level, and vice versa.
[3]Average of ratings based on: 1. essentially tap rooted, 2. moderately branched, 3. abundantly branched.
[4]Obtained by multiplying grams root weight by per cent total available carbohydrates.

All of the plants in T1 where they were allowed to grow and flower freely had flowering stems by early October when classified for plant types (Table 4). An average of 28 per cent were type 3, 40 per cent type 4, and 32 per cent type 5. Plant types 4 and 5 were characterized by weak or virtually no crown rosette of leaves and tillers and had upright flowering stems.

Treatments that prevented flowering changed the plant type composition of the populations in a marked summer. In T2, where the flower buds were removed at weekly intervals until early October, all of the plants had flowering stems by early October as in T1. However, there was a shift in plant types toward a higher proportion of the flowering plant type (type 3) with broader crowns and more vegetative development. A large percentage of the plants in T3, T4, and T5 consisted of plants with few or no flowering stems when classified in early October. This, in part, was the result of defoliation and stem cutting that removed flowering stems prior to the classification of plant types. Even so, the plants in these treatments produced very large, well-developed, leafy crown rosettes.

Differentials in winterkilling among the treatments were apparent following the winters of 1956-57 and 1957-58, even though severe elimination of plants occurred during the winter of 1956-57. Virtually all plants were killed during the winter of 1958-59. Plant counts following the winters of 1956-57 and 1957-58 (Table 4) indicated that treatments that prevented flowering improved survival over winter. The poorest survival occurred in T1 where the plants grew and flowered freely during the seedling year. The best winter survival occurred in T3 where, at weekly intervals during the summer, the tillers were removed close to the crowns as they began to elongate into stems.

The average number of non-flowering tillers, width of the crown, and dry weight of the crown per plant in the late fall of the seeding year were significantly higher for the treated plants than for the untreated plants (Table 5). The number of non-flowering tillers per plant varied from a low of 8 in the untreated plants (T1) to a high of 16 in T3 and T5. Plants in T2 and T4 were intermediate in tiller numbers. The width of the crowns varied from a low of 8.2 inches per plant in T1 to a high of 15.1 inches in T3. Plants in T2, T4, and T5 were intermediate in crown width and were significantly smaller than in T3. The dry weight of the crowns varied from a low of 5.9 grams per plant in T1 to a high of 24.0 grams per plant in T3. Plants in T2, T4, and T5 were intermediate in crown weight and were significantly lower in weight than in T3.

Roots, like the above-ground parts, were greatly modified by the treatments. The differences occurred particularly in the type, dry weight, and amount of carbohydrates. The roots of all the treated plants had significantly more branching than the roots of the untreated plants. The latter were more or less tap rooted with little or no branching. There was no significant difference in root type among the plants in T2, T3, T4, and T5, but the root systems of the plants in T2 and T3 had more lateral branches.

The dry weight of the roots varied from a low of 4.1 grams per root in T1 to a high of 7.7 grams in T2. The dry weight of the roots in T2 and T3 were similar, and both were significantly greater than that of T1. The root weights in T4 and T5 were intermediate, but did not differ significantly from that of T1. The amount of total available carbohydrates in the roots in grams followed in the same order as the root weights for the various treatments. The percentage of total available carbohydrates in the roots was lowest in the untreated plants but differences among the other treatments were small.

Dollard was used along with Wisconsin Common in seedings made in 1958. The response of Dollard to the various treatments was the same as for Wisconsin Common except that Dollard, as expected, showed more vegetative vigour.

## DISCUSSION

Seedings made at five different dates at 2-week intervals from May 15 to July 15 showed that the percentage of flowering plants in spaced populations of several clovers was highest at the earliest seeding date and declined with later seeding dates. No plants flowered in the July 15th seedings. These results are in agreement with those of Smith (9). As reported previously by others (2, 4, 9), a higher percentage of winterkilling occurred in the flowering plants of each clover than in the non-flowering plants.

Almost all of the plants of Wisconsin Common, Lakeland, and Dollard medium red clover, and of alsike clover, produced flowering stems when seeded in mid-May. The percentage of flowering plants at each seeding date for the medium red clover varieties was lowest in Dollard and highest in Wisconsin Common, while Lakeland was intermediate. Only a very small percentage of the plants of Altaswede mammoth red clover produced flowering stems even at the earliest seeding date. Winter survival over all dates of seeding was highest in Altaswede followed in order by alsike, Dollard, Lakeland, and Wisconsin Common.

Prevention of flowering by removing flower buds, flowering stems, and/or elongating crown tillers markedly influenced the growth pattern of medium red clover plants. Plants that were allowed to flower freely had a smaller number of non-flowering crown tillers, a smaller width of crown, a lower dry weight of crown, root and total available carbohydrates in the roots, less root branching, and a slightly lower percentage of total available carbohydrates in the roots than plants that were prevented from flowering. The winter survival of the plants allowed to flower freely also was lower than for the plants that were prevented from flowering. Plants where crown tillers were removed as they began to elongate at weekly intervals during the summer until August 31 had the greatest development of crown and root and the highest percentage of winter survival. There was little difference among treatments in the percentage of total available carbohydrates in the roots where flowering was prevented. Cumming (3) also obtained a greater development of lateral buds on medium red clover plants through the cessation of growth and of differentiation of the floral apices by the application of certain growth regulators.

The improvement of winter survival through the prevention of flowering allows a clearer understanding of why seedling stands of medium red clover usually are improved by clipping. Willard and Lewis (11), Willard (12), Allen and Kuhn (1), and Klebesadel and Smith (7) have shown that clipping medium red clover following harvest of the grain companion crop markedly improved stands and yields the following year. It has been suggested that the presence of the grain stubble and weeds might aid in the development of high humidity conditions near the soil surface which would increase the incidence of certain clover diseases. The current results appear to indicate that the important factor may be that clipping removes the elongating tillers and flowering stems. This holds the plants in a vegetative condition that results in greater crown and root development and improved winter survival.

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# INHERITANCE OF REACTION TO SEVERAL RACES OF CROWN RUST, *PUCCINIA CORONATA AVENAE* ERIKSS., IN TWO CROSSES INVOLVING UKRAINE OATS[1]

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## ABSTRACT

The oat variety Ukraine is resistant to a large number of crown rust races, including race 263, a race which is pathogenic on almost all commercial oat varieties in North America. Random $F_3$ lines from the crosses Fortune x Ukraine and Garry x Ukraine were tested for their reaction to races 213 and 263 of crown rust, and lines selected from these were tested for reactions to other races. The tests indicated that a single dominant gene in Ukraine governed resistance to races 213, 202, 202A, 209, 212A, 235, 236, 239, 239B, and 240, and that another dominant gene in Ukraine governed resistance to race 263. Tests with lines from one family of the Garry x Ukraine cross showed that the reactions of Garry to races 229, 235, and 240 are associated in inheritance, and tests with lines from a second family showed that the reactions to races 209, 236, and 239 are also associated in inheritance. The data also indicate that one or more genes in Garry govern resistance to these races, but whether or not the same gene or genes govern resistance to both groups of races was not determined. The Ukraine and Garry rust reactions were shown to be inherited independently of one another, with the gene for the higher type of resistance epistatic to the gene for the lower type in all cases.

A number of $F_3$ lines were isolated which combined the field resistance of Ukraine and Garry, respectively, to crown rust, including race 263, with the field resistance to stem rust of Garry. These lines may prove very useful in a plant breeding program.

## INTRODUCTION

Crown rust or leaf rust of oats, caused by *Puccinia coronata avenae* Erikss., occurs in most countries where oats are grown. According to Simons and Murphy (8) it is the most serious disease of oats in the eastern half of the United States. Peturson (6) reported that crown rust often causes severe crop losses in Eastern Canada, and in Manitoba and eastern Saskatchewan.

The control of crown rust has been of great economic importance in Canada and the United States, and the main control used has been the development of varieties resistant to the pathogen.

Simons (7) reported a new race of crown rust in 1954, identified as race 263, which vigorously parasitized most oat varieties formerly used as sources of resistance. Ukraine is the best known source of resistance to this race, and if it becomes prevalent the Ukraine resistance will be of great value.

This study was undertaken, therefore, to determine (a) the mode of inheritance of the Ukraine reaction to race 263 and other races of crown rust, (b) the mode of inheritance of the Garry reaction to certain races, and (c) the degree of association between the inheritance of factors for resistance in Ukraine and Garry.

[1]Contribution No. 23, Research Branch, Canada Department of Agriculture Research Station, Winnipeg, Man., from a thesis submitted to the University of Manitoba in partial fulfilment of the requirements for the degree of Master of Science.

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## MATERIALS AND METHODS

The varieties used as parents in this study were as follows:

(1) *Ukraine* was introduced into the United States from Russia in 1930 and into Canada from the United States in 1945. This variety is valuable in breeding for crown rust resistance, particularly for race 263.

(2) *Garry* is from the cross Victory x Victoria x (Hajira-Banner). The original Garry, developed at the Cereal Breeding Laboratory, Winnipeg, Man., was released in 1947. It was withdrawn and replaced in 1952 by a new selection, R.L. 1692.27, which was resistant to Victoria blight, but susceptible to some races of crown rust. It was this selection that was used in this study.

(3) *Fortune*, developed at the University of Saskatchewan, is from the cross Victory x [(Victoria-Richland) x (Markton-Victory)].

The rust reactions of the three parental varieties and of Rodney, an accessory host for identification of races, are shown in Table 1.

The two crosses studied, namely Garry x Ukraine and Fortune x Ukraine, were made in the greenhouse at the Cereal Breeding Laboratory

TABLE 1. — SEEDLING REACTION OF PARENT VARIETIES AND RODNEY TO 12 RACES OF CROWN RUST

| Races of Crown Rust | Varieties | | | |
|---|---|---|---|---|
| | Ukraine | Garry | Fortune | Rodney |
| 202A | R | S | S | S |
| 212A | R | S | S | S |
| 213 | R | S | S | S |
| 239B | R | S | S | S |
| 263 | R | S | S | S |
| 202 | R | R | S | R |
| 209 | R | R | S | R |
| 235 | R | R | S | R |
| 236 | R | R | S | R |
| 239 | R | R | S | R |
| 240 | R | R | S | R |
| 229 | S | R | S | R |

TABLE 2. — GOODNESS OF FIT TO A 1:2:1 RATIO OF $F_3$ LINES FOR REACTION TO RACE 213 OF CROWN RUST IN CROSSES OF RESISTANT UKRAINE WITH SUSCEPTIBLE FORTUNE AND GARRY

| Cross | Classes | | | | | | Chi-square | P |
|---|---|---|---|---|---|---|---|---|
| | Resistant | | Segregating | | Susceptible | | | |
| | Obs. | Exp. | Obs. | Exp. | Obs. | Exp. | | |
| Fortune x Ukraine | 24 | 21.50 | 40 | 43.00 | 22 | 21.50 | 0.61 | 0.70-0.80 |
| Garry x Ukraine | 40 | 34.25 | 70 | 68.50 | 27 | 34.25 | 2.53 | 0.20-0.30 |
| Total | 64 | 55.75 | 110 | 111.50 | 49 | 55.75 | 2.04 | 0.30-0.50 |

at Winnipeg during the winter of 1954-55. Seven $F_1$ plants from each cross were grown in the field at Winnipeg in 1955, together with the parents of each $F_1$ plant. Three families of each cross and their parents were grown in the field at the Central Experimental Farm, Ottawa, in 1956. All tests on crown rust reaction were made in the greenhouse of the Plant Science Department, University of Manitoba, during the winter of 1956-1957, and the greenhouse of the Cereal Breeding Laboratory in the spring and summer of 1957.

*Techniques of Testing Lines*

In this paper the term "family" is used to refer to a group of $F_2$ plants or $F_3$ lines derived from a single $F_1$ plant. The term "line" or "$F_3$ line" refers to the progeny of a single $F_2$ plant. Sixty to eighty $F_3$ lines from each family were taken at random to determine their reaction to races 263 and 213. Groups of 30 lines were selected on the basis of their reaction to these races for testing to other races of crown rust.

For these tests approximately 30 seeds per pot were sown when testing to a single race, and 15 seeds when testing to a number of races. In some of the tests to a number of races, the method described by Knott and Anderson (5), in which the plants were inoculated in the one-leaf stage with one race and in the second-leaf stage, 7 to 8 days later, with another race, was used. The seedlings were classified according to pustule type, and on this basis the lines were then classified as resistant, segregating, or susceptible. In all cases the parents were used as checks. The inoculum was applied by hand and, following a 24-hour incubation period, the greenhouse was kept at a temperature of approximately 70°F. during the day and at a reduced temperature during the night.

Pure races were obtained from B. Peturson, of the Plant Pathology Laboratory, Winnipeg. These races were classified on the set of ten differentials suggested by Simons and Murphy (8) with Rodney and Garry used as accessory hosts. Several of the races used were sub-races differentiated in Canada on the basis of these two accessory hosts. The purity of races was usually checked on the differential hosts both before and during tests.

The data were statistically analysed using the Chi-square test, and the correction for continuity was applied in all cases where there was only one degree of freedom.

## EXPERIMENTAL RESULTS

*Tests with Race 213*

Ukraine is almost immune to this race while Garry and Fortune are completely susceptible. A total of 86 random $F_3$ lines from the cross Fortune x Ukraine and 137 random $F_3$ lines from the cross Garry x Ukraine were classified as resistant, segregating, or susceptible to race 213, and the data fitted to a 1:2:1 ratio. The data from these tests, presented in Table 2, indicate that in both of these crosses a single factor is operative, as shown by P values of 0.70 — 0.80 and 0.20 — 0.30, respectively. The P value for the totals of both crosses is 0.30 — 0.50.

TABLE 3. — GOODNESS OF FIT TO A 1:2:1 RATIO OF $F_3$ LINES FOR REACTION TO RACE 263 OF CROWN RUST IN CROSSES OF RESISTANT UKRAINE WITH SUSCEPTIBLE FORTUNE AND GARRY

| Cross | Classes | | | | | | Chi-square | P |
|---|---|---|---|---|---|---|---|---|
| | Resistant | | Segregating | | Susceptible | | | |
| | Obs. | Exp. | Obs. | Exp. | Obs. | Exp. | | |
| Fortune x Ukraine | 13 | 17 | 38 | 34 | 17 | 17 | 1.44 | 0.30-0.50 |
| Garry x Ukraine | 32 | 28 | 62 | 56 | 18 | 28 | 4.78 | 0.05-0.10 |
| Total | 45 | 45 | 100 | 90 | 35 | 45 | 3.33 | 0.10-0.20 |

TABLE 4. — FREQUENCY DISTRIBUTION SHOWING THE RELATIONSHIP BETWEEN THE SEEDLING REACTION OF $F_3$ LINES TO RACES 213 AND 263 OF CROWN RUST IN TWO CROSSES

| Cross | Reaction to Race 263 | Reaction to Race 213 | | | Total |
|---|---|---|---|---|---|
| | | Res. | Seg. | Susc. | |
| Fortune x Ukraine | Res. | 12 | 1 | 1 | 14 |
| | Seg. | 6 | 28 | 0 | 34 |
| | Susc. | 0 | 0 | 16 | 16 |
| | Total | 18 | 29 | 17 | 64 |
| Garry x Ukraine | Res. | 20 | 4 | 1 | 25 |
| | Seg. | 4 | 48 | 1 | 53 |
| | Susc. | 0 | 0 | 16 | 16 |
| | Total | 24 | 52 | 18 | 94 |

*Tests with Race 263*

The reaction of the parental variety Ukraine varied in different tests from a resistant (type 1) pustule to a moderately susceptible (type 3) pustule, while the parents Garry and Fortune gave susceptible (type 4) pustules. A total of 180 random $F_3$ lines, consisting of 68 lines from the cross Fortune x Ukraine and 112 lines from the cross Garry x Ukraine, were classified as resistant, segregating, or susceptible to race 263 and fitted to a 1:2:1 ratio. The data presented in Table 3 indicate that in both the Fortune x Ukraine and Garry x Ukraine crosses a single gene conditions resistance to race 263, as shown by P values of 0.30 – 0.50 and 0.05 – 0.10, respectively. The P value for the totals of both crosses is 0.10 – 0.20.

*Association Between Inheritance of Reaction to Races 213 and 263*

The relationship between the inheritance of reaction to races 213 and 263 was determined for 64 random $F_3$ lines of the Fortune x Ukraine cross

and for 94 random $F_3$ lines of the Garry x Ukraine cross. The majority of the lines which were classified as resistant, segregating, or susceptible to one race gave a similar reaction to the other race, as indicated in Table 4.

Eighteen lines did not follow this pattern. Two of them were classified as susceptible to race 213 and resistant to race 263. Ten lines were resistant to race 213 and segregated for reaction to race 263, while five lines segregated for reaction to race 213 and were resistant to race 263. One line was susceptible to race 213 and segregated for reaction to race 263. Although the data presented up to this point indicate a single gene for crown rust resistance in the variety Ukraine, the reactions of the lines mentioned above do not conform with those expected on the basis of this hypothesis.

There are two possible explanations of these results: either the reactions varied due to environment, or they were conditioned by two closely-linked genes. Both conditions, of course, may be involved. Although the reactions brought about by race 213 do not appear to be unduly affected by environment, those associated with race 263 are more subject to modification.

In view of the possibility of two closely-linked genes being involved it is of interest to estimate the theoretical crossover value which would conform with the results in Table 4. It was not possible to estimate the linkage intensity using the product method, since the sum of the classes making up the factor "b" was equal to zero in both crosses; therefore the method of maximum likelihood as applied to $F_2$ and $F_3$ data (4) was used.

There was a marked inconsistency of results when the maximum likelihood method was applied to different portions of the data. Estimates of crossing-over were as follows: from deduced $F_2$ data, 2 per cent; from singly dominant $F_2$ plants carried to $F_3$, 4 per cent; and from doubly dominant $F_2$ plants carried to $F_3$, 17 per cent. The estimate from the three methods combined was 11 per cent. However, the Chi-square test for goodness of fit to frequencies calculated on a basis of 11 per cent crossing-over gave a P value of less than 1 per cent. On the whole the evidence suggests two rather closely-linked genes and a considerable variation of rust reactions due to environment.

TABLE 5. — GOODNESS OF FIT TO A 3:1 RATIO OF PLANTS WITHIN SEGREGATING $F_3$ LINES FOR REACTION TO RACES 202, 202A, 209, 236, AND 239 OF CROWN RUST IN A CROSS OF RESISTANT UKRAINE WITH SUSCEPTIBLE FORTUNE

| Race | Resistant | | Susceptible | | Chi-square | P |
|---|---|---|---|---|---|---|
| | Obs. | Exp. | Obs. | Exp. | | |
| 202 | 141 | 141.75 | 48 | 47.25 | 0.01 | 0.99-1.00 |
| 202A | 108 | 108.00 | 36 | 36.00 | 0.00 | 1.00 |
| 209 | 134 | 127.50 | 36 | 42.50 | 1.03 | 0.30-0.50 |
| 236 + 239 | 122 | 131.25 | 53 | 43.75 | 2.33 | 0.10-0.20 |
| Total | 505 | 508.50 | 173 | 169.50 | 0.06 | 0.80-0.90 |

*Tests of Several Races on Selected Lines*

1. *Fortune x Ukraine*

Ukraine is resistant and Fortune is susceptible to races 202, 202A, 209, 213, 236, and 239. Thirty $F_3$ lines from one family of this cross were selected on the basis of their reaction to race 213. Nine lines were resistant to this race, nine segregated for reaction, and twelve were susceptible. These lines were tested for their reaction to races 202, 202A, 209, 236, and 239. All lines classified as resistant, segregating, or susceptible to race 213 gave a similar reaction to the other five races. These results indicate that the single dominant gene in Ukraine which conditions resistance to race 213 also conditions resistance to races 202, 202A, 209, 236, and 239.

In all lines classified as segregating the data showed a good fit to the expected ratio of 3 resistant to 1 susceptible (Table 5). This provided further evidence in favour of the hypothesis that a single dominant gene in Ukraine conditions resistance to the races of crown rust studied.

2. *Garry x Ukraine*

(a) Thirty $F_3$ lines from one family of this cross were selected on the basis of their reaction to race 213. Eleven lines were resistant to this race, ten segregated for reaction, and nine were susceptible. These lines were inoculated with races 202A, 212A, 239B, and 263, to which Ukraine is resistant; race 229 to which Garry is resistant; and races 235 and 240 to which both parents are resistant. The results from these tests are discussed below:

i) *Races 202A, 212A, and 239B.* In all cases the reaction of the 30 lines to these races was the same as their reaction to race 213. This indicates that the Ukraine reaction to all four races is governed by the same gene.

ii) *Race 263.* In most cases the reaction of the lines to race 263 was the same as their reaction to race 213. However, two lines which were resistant to race 213 segregated for reaction to race 263 in two separate tests. The data support the hypothesis that the gene for resistance to race 263 is closely linked with the gene for resistance to race 213.

iii) *Race 229.* The reaction of the lines to this race differed from their reaction to race 213. This indicates that the gene or genes from Garry for resistance to this race are independent from the Ukraine gene.

iv) *Races 235 and 240.* All lines which showed either the Ukraine resistance to race 213 or the Garry resistance to race 229 were resistant to races 235 and 240. The three lines which were susceptible to races 213 and 229 were also susceptible to races 235 and 240. The remainder of the lines segregated for reaction to these two races. These results indicate that the Ukraine gene for resistance to race 213 controls resistance to races 235 and 240, and that the Garry gene or genes for resistance to race 229 also control resistance to races 235 and 240.

(b) Thirty $F_3$ lines from a second family of the cross Garry x Ukraine were selected on the basis of their reaction to race 213. Nine lines were resistant to this race, thirteen segregated for reaction, and eight were susceptible. These lines were inoculated with races 202A and 263 to which Ukraine is resistant and with races 209, 236, and 239 to which both parents are resistant. The results from these tests are discussed below:

i) *Race 202A.* The reactions of the 30 lines tested to this race were the same as their reaction to race 213. This indicates that the Ukraine resistance to race 213 and to race 202A is governed by the same gene.

ii) *Race 263.* In most cases the reaction of the 30 lines to race 263 was the same as their reaction to race 213. However, four lines were exceptions to this pattern. One line resistant to race 213 segregated for reaction to race 263. One line which

segregated to race 213 was resistant to race 263, and of two lines which were susceptible to race 213, one segregated and one was resistant to race 263. The classification of these 4 lines to race 263 was based on three or more separate rust tests all giving the same reaction. These data lend support to the hypothesis that the gene for resistance to race 263 is closely linked with the gene for resistance to race 213. As the data were obtained from lines selected on the basis of crown rust reaction rather than from random lines, an analysis of linkage intensity was not made.

iii) *Races 209, 236, and 239.* All 30 lines gave similar reactions to these races. The 9 lines that were resistant to race 213 gave the Ukraine type of resistance to these three races. All 13 lines that segregated for reaction to race 213 segregated or were resistant to these races, while the eight lines susceptible to race 213 were resistant, segregated, or were susceptible to the three races. This indicates that the Ukraine gene for resistance to race 213 also controls resistance to races 209, 236, and 239; and in addition that the Garry gene or genes control resistance to the same three races.

## DISCUSSION

These investigations were undertaken with the assumption that the parental varieties were homogeneous for crown rust reaction. Rust tests were made on hybrid progenies from four parental plants of Ukraine and two parental plants of Garry. As there did not appear to be a significant variation in results between different families of the same cross, no evidence was found of heterogeneity for crown rust reaction.

Finkner *et al.* (2) stated that a single dominant gene in Ukraine gives resistance to race 57 and that this Ukraine gene conditions susceptibility to race 109, even in the presence of the Bond gene for resistance to this race. The present study agrees with the results of Finkner *et al.* in finding a single gene for resistance to race 202A (new designation for race 57). No crosses with Bond were made, but it was found that the Ukraine gene did not inhibit the Garry gene for resistance to race 229 (new designation for race 109). Finkner (3) concluded that Ukraine resistance to race 57 is controlled by duplicate dominant linked genes rather than by a single dominant gene as found in this study. This difference in results could be explained by the fact that the original Ukraine stock was not homogeneous for crown rust reaction and, therefore, genetically different Ukraine plants may have been used as parents in these two investigations.

Finkner (1) and Finkner (3) reported a gene in Victoria for resistance to race 57 and susceptibility to Victoria blight. Welsh *et al.* (9) indicated that this gene is lacking in Garry, but that Garry has a single dominant gene for resistance to a large number of races. This gene governs resistance to races 2, 29, and 28 (which are now grouped as race 239); 3 and 24 (grouped as race 240); and 2b (now designated 229). The present investigation revealed the reactions of Garry to races 229, 235, and 240 to be associated in inheritance, and the reactions to races 209, 236, and 239 to be also associated in inheritance. Resistance to the races used in this study is probably conditioned by the same gene as that studied by Welsh *et al.* (9) which was shown to condition resistance to races 229, 239, and 240; therefore, the same gene must give resistance to all six races.

Finkner (1) and Finkner (3) found allelism between the gene or genes in Ukraine and the gene or genes in Santa Fe, Trispernia, and Bond. The Ukraine gene was independent of genes in Landhafer and Victoria. In

this investigation only the Ukraine gene and the Garry gene were studied. This Garry gene is not the same gene as that controlling resistance to race 57 (202A) in Victoria, and its allelic relationships had not been previously studied. It was found to be independent of the Ukraine gene.

A number of lines from the Garry x Ukraine cross possess the seedling resistance to crown rust of both parents. Some of these lines, selected in the field, possessed the crown rust resistance of both parents and the stem rust resistance of Garry. Such lines will be valuable as breeding material.

Field reactions determined at Winnipeg in 1957 under artificially induced epidemic conditions show that in that year the Ukraine resistance to the races of crown rust prevalent in Western Canada was equal to that of Landhafer and Santa Fe. Ukraine also controls resistance to one or more of the Landhafer races in the seedling stage. Finkner *et al.* (2) and Finkner (3) indicated that the genes for resistance in Ukraine and Santa Fe are allelic and, therefore, the resistance from both sources cannot be combined in one genotype. The Ukraine and Landhafer genes, on the other hand, were shown by Finkner (3) to be independent of one another and, therefore, the resistance of these two varieties can readily be combined. The changes in race population in the crown rust organism over the past few years have been very rapid and great effort will be required to maintain commercial varieties resistant to the prevalent races of this pathogen.

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# INHERITANCE OF SEED WEIGHT AND ASSOCIATED TRAITS IN BROMEGRASS, *BROMUS INERMIS* LEYSS.[1]

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## ABSTRACT

Among 71 $S_0$ and $S_2$ clones of diverse origin in a space-planted, replicated polycross nursery the range in mean seed weight was 0.71 to 1.30 grams per 300 seeds. A correlation of 0.25 was noted between seed weight and open-pollinated seed set for these clones. Their polycross progenies exhibited a range of only 0.74 to 0.94 grams per 300 seeds in a solid-planted test. The progeny-parent regression value was non-significant ($b = 0.09$) while the correlation ($r = 0.56$) was significant at the 1 per cent level.

Inbred segregates of 10 representative clones were evaluated in a replicated test for seed weight, fertility, seed yield and bloom date. In each family, segregates showed significant differences in performance for the former three traits which is indicative of genetic segregation. These three traits all exhibited substantial inbreeding depression, averaging 21, 20 and 53 per cent for seed weight, fertility and seed yield, respectively, from $S_0$ to $S_1$ for seven families, and 19, 24 and 66 per cent, respectively from $S_2$ to $S_3$ for three families. Inbreeding resulted in a delay of 1 to 2 days in bloom date, on the average.

Progeny-parent regression and correlation values for the four traits were all significant. The regression value for seed weight, 0.39, was equal to that for yield but lower than that of fertility, 1.19, or bloom date, 0.78. Correlations ranged from 0.83 to 0.94. Seed weight, fertility index and seed yield all were positively associated in intercharacter correlation studies, indicating no adverse relationships from a selection standpoint.

## INTRODUCTION

In the breeding of a number of perennial forage grasses increased seed weight appears to be a worthwhile objective. Development of heavier seeded types should enhance stand establishment, improve seed handling ease, and possibly increase seed yields. Although forage production is the primary consideration in a breeding program, seed production must also be considered, since seed is the cheapest method of propagation in most species. The present study was part of a series undertaken to explore possibilities of breeding new varieties of bromegrass with heavier seed. For this purpose a number of selected bromegrass clones and their polycross and inbred progenies were evaluated to study the extent of variability for seed weight and several other possible associated seed yield traits and to estimate heritabilities. Also of interest were relationships between seed weight and these other seed traits, effects of inbreeding, and extent of segregation in the $S_1$ generation. Results are presented and discussed with these objectives in mind.

## REVIEW OF LITERATURE

There are few published estimates of amount of genotypic variability for seed weight existing within forage species or varieties. Significant differences in clonal or strain performance for this trait have been reported in several native range grasses (5, 6), in bromegrass (10, 13), and in intermediate wheatgrass (4, 8). It would appear, therefore, that opportunities for improvement of seed weight exist in these forage species.

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Other seed yield components have been studied more extensively. Grennell (3) studied variation among a number of bromegrass clones of "desirable" and "undesirable" phenotypes. Estimated percentages of genotypic variance on a plant-mean basis in the two groups were 94 and 91 per cent for seed yield and 94 and 96 per cent for fertility, respectively. Truscott (14) estimated per cent genotypic variance among spaced plants within a number of bromegrass strains of differing origin. For seed yield, panicle rating and fertility index, estimates were 39, 16 and 68 per cent, respectively. Genotypic variation determined among spaced plants, however, may not be indicative of relative performance in solid stands. In this regard, Thomas and Kernkamp (12) reported estimates of 0 and 18 per cent for seed yield based upon data from a polycross test grown at two locations in Minnesota.

Nielsen and Kalton (9) studied combining ability in bromegrass for the characters: seed yield, panicle production, fertility and seed weight. Topcross progenies of 18 $S_0$ clones and of 10 $S_1$'s from each were evaluated. Differences among families were significant for all traits studied. In addition, evidence of segregation for combining ability was found in 17, 15 and 11 families for seed yield, panicle production and fertility, respectively. For seed weight, significant differences among $S_1$ topcross progenies were found in the four families studied. Regression of mean $S_1$ topcross performance on $S_0$ topcross performance gave coefficients of 0.32, 0.38, 0.67 and 0.83 for seed yield, panicle production, seed weight, and fertility, respectively.

Knowles (7) studied seed yields of a number of bromegrass clones in spaced plantings and of their open-pollination progenies in spaced or row plantings. Progeny-parent regressions were low and non-significant. Non-significant progeny-parent correlations were reported by Thomas and Kernkamp (12) in a study of seed yields of parental clones grown in spaced plantings and of their polycross progenies in solid rows. Raeber and Kalton (11) reported a heritability of 49.4 per cent for fertility in bromegrass, based upon the regression of mean $S_1$ performance on $S_0$ mean in eight families. In sand bluestem, Kneebone (5) found a low but significant correlation ($r = 0.34$) between mean seed weights of a number of clones and those of their open-pollination progenies.

Wilsie *et al.* (15) studied fertility of 26 $S_0$ bromegrass clones and of one $S_1$ and one $S_2$ selection from each. Average numbers of seeds per open-pollinated panicle were 126, 114 and 102 for $S_0$, $S_1$ and $S_2$ generations, respectively. Nielson and Kalton (9) concluded that inbreeding is of little value as a means of obtaining plants superior in combining ability for seed yield or its major components.

Although seed weight is a component of seed yield, Nielson and Kalton (9) and Truscott (14) concluded that its contribution to total seed production is less than that of fertility or panicle production.

## MATERIALS AND METHODS

Materials used to initiate this study consisted of 71 agronomically desirable clones under investigation in the grass breeding program of the

Iowa Agricultural Experiment Station. None, however, had undergone any previous evaluation for seed weight. Of the 71 clones, 44 were $S_0$ and $S_2$ selections of northern and intermediate origin while the remaining 27 were of southern origin. In 1951 these clones were established in a space-planted polycross nursery consisting of six replications of three-plant plots. In 1952, several panicles on the centre plant in each plot were selfed while the remaining ones were allowed to open-pollinate. Plants were evaluated for self-fertility by counting the number of seeds per self-pollinated panicle. For open-pollinated fertility, a fertility index was obtained from weight of clean seed per two panicles divided by total weight of the two unthreshed panicles and multiplied by 100. Raeber and Kalton (11) suggested the use of such an index for fertility evaluations.

To form a composite polycross seed lot for each clonal entry, equal weights of seed were taken from each replication and bulked. From each of these seed lots, four random samples of 300 seeds each were counted and weighed to the nearest .01 grams on a torsion balance. Seed weight data were subjected to a statistical analysis and correlations were calculated among seed weight, number of selfed seeds per panicle and open-pollinated fertility index, using mean values for each clone.

The bulk polycross seed lots also were used to plant a polycross progeny test in 1953 which was harvested for forage in 1954 and 1955. In 1956 this solid-planted test was harvested for seed yield determinations and the resulting seed evaluated for seed weight. For this purpose, 150 seeds were counted from the cleaned seed obtained from each of the five replications of each entry. These samples were weighed in the same manner as those for the parental clones.

For the inbreeding phase of the study, 10 clones from the polycross nursery were selected to represent the total range in mean seed weight. Four were high, three low, and three of intermediate or average seed weight. Of these 10 clones, 7 were non-inbred ($S_0$'s) and of southern origin, while 3 had been inbred for two generations ($S_2$'s) and were of northern origin. Inbred progenies of these selections were established in the greenhouse during the winter of 1956-57 and in May, 26 inbred plants from each of the 10 progenies were divided vegetatively into three propagules. Later, all plants were transplanted to the field in a randomized complete block design, including a split-plot arrangement, with three replications. Each whole plot (family) consisted of single-plant sub-plots of propagules of 26 inbred plants of a family plus a propagule of the parental clone, all space-planted and arranged in a randomized order. These will be referred to as "inbred" progeny and "clonal" progeny, respectively. There were 10 whole plots in each replication and each plant entry was represented by one propagule in each replication. With one exception, all families became well-established. In August, 1957, the experiment was overseeded with alfalfa to control weeds, prevent excessive spreading of the plants and provide nitrogen. Resulting stands of alfalfa were excellent and served their purpose well.

In 1958, entries were evaluated on an individual plant basis for bloom date, fertility index, seed yield and seed weight. Bloom date was recorded

as date on which anthesis began while fertility indices were calculated as previously described. Total weight of clean seed yielded by a plant was recorded to the nearest gram. For seed weight, two samples of 50 seeds each for each plant were weighed to the nearest milligram on a precision balance. A previous study, reported elsewhere (1), had indicated that this method gives a reasonably precise determination for a low relative cost.

In statistical analysis of these data, separate analyses of variance and covariance were computed for each family. Variances and covariances among inbred plants within families were separated into genotypic and environmental components and these were used to calculate per cent genotypic variance for each trait and the simple, phenotypic, genotypic and environmental correlations. Simple correlations among seed weight, fertility index and seed yield were then used to calculate partial correlations among these traits.

Heritabilities for each trait were estimated by means of progeny-parent regressions and correlations. In addition, per cent inbreeding depression was calculated by means of the following formula:

$$\frac{\bar{P} - \bar{I}}{\bar{P}} \times 100$$

where $\bar{P}$ = mean of the clonal progeny and $\bar{I}$ = mean of the inbred progeny.

## EXPERIMENTAL RESULTS

### PARENTAL CLONE PERFORMANCE

Mean seed weights for the 71 clones in the polycross nursery ranged from 0.71 to 1.30 grams per 300 seeds. The frequency distribution of these means is illustrated graphically in Figure 1. The mean of the entire population was 1.00 grams per 300 seeds and the distribution approached normality. As might be expected on the basis of this distribution, mean differences among clones were found to be significant (1 per cent level) in the analysis of variance. The coefficient of variation (C.V.), based upon sampling error, was 2.2 per cent indicating a relatively precise evaluation.

Clonal means for number of seeds per self-pollinated panicle ranged from 0 to 128.1 with a grand mean of 20.5 seeds. Mean O.P. fertility indices for the 71 clones exhibited a range of 18 to 71 per cent and the grand mean was 55 per cent. These data were not analysed statistically. Mean seed weight showed a negative but non-significant association ($r = -0.12$) with mean selfed seed set and a positive association ($r = 0.25$, significant at the 5 per cent level) with mean open-pollinated fertility index. Between selfed seed set and open-pollinated fertility the correlation was 0.34 and was significant at the 1 per cent level. The partial correlation between seed weight and open-pollinated fertility, 0.31, was significant at the 1 per cent level. These correlations between seed weight and fertility are encouraging when viewed from a selection standpoint.

### POLYCROSS PROGENY TEST

Among the polycross progenies, the range in mean seed weight was 0.72 to 0.94 grams per 300 seeds with a grand mean of 0.80 grams. Both the range and the test mean were less than those for the parental clones.

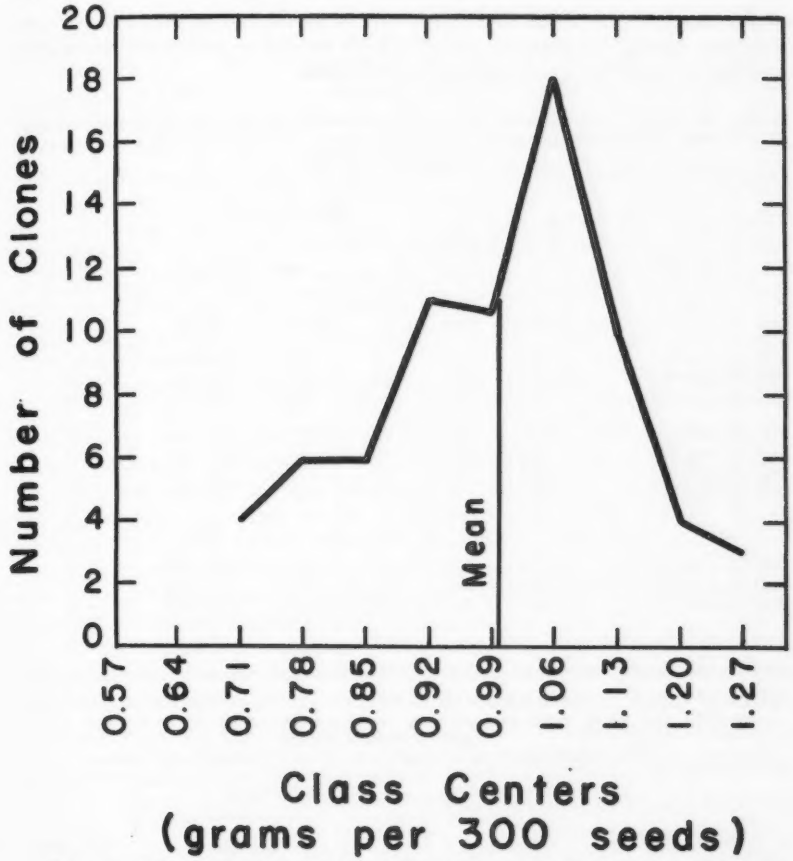


FIGURE 1. Mean seed weight distribution for polycross seed of 71 $S_0$ and $S_2$ clones.

The reduction in range would be expected because of the nature of the progenies, while the reduction in the grand mean was probably due in part to the solid planting and the very dry conditions prevailing in 1956. In the analysis of variance of the data, the mean square for progenies was significant at the 1 per cent level. This indicates significant differences among the clones in combining ability for seed weight. The C.V. in this analysis, based upon experimental error rather than sampling error, was only 5 per cent. From this it might be concluded that one sample of 150 seeds per replication was sufficient to accurately evaluate progenies for seed weight differences in solid-seeded plots.

An estimate of heritability was obtained from regression of progeny on parental means. For mean seed weight in this material the regression value was quite low ($b = 0.09$), while the correlation between progeny and parental means was 0.56 and significant at the 1 per cent level. The

difference between these two values can be attributed to the relatively low variance among the progeny means which tended to inflate the correlation coefficient relative to the regression coefficient.

TABLE 1.—MEAN SEED WEIGHTS OF 10 SELECTED CLONES IN THE POLYCROSS NURSERY IN 1952 AND MEAN PERFORMANCE FOR SEED WEIGHT OF THEIR CLONAL AND INBRED PROGENIES IN 1958

| Family | Parental clone mgm./50 seeds | | Inbred progeny | | | Inbreeding depression (%) |
|---|---|---|---|---|---|---|
| | | | No. of plants | mgm./50 seeds | | |
| | 1952 | 1958 | | Mean | Range | |
| 471 ($S_0$ and $S_1$) | 217 | 236 | 26 | 159 | 97–209 | 32 |
| 383 ($S_0$ and $S_1$) | 187 | 234 | 17 | 169 | 107–216 | 28 |
| 547 ($S_0$ and $S_1$) | 187 | 195 | 26 | 156 | 116–195 | 20 |
| 521 ($S_0$ and $S_1$) | 165 | 203 | 25 | 151 | 111–186 | 26 |
| 613 ($S_0$ and $S_1$) | 160 | 178 | 26 | 145 | 113–168 | 18 |
| 567 ($S_0$ and $S_1$) | 125 | 152 | 26 | 124 | 91–178 | 18 |
| 533 ($S_0$ and $S_1$) | 123 | 157 | 26 | 147 | 97–171 | 6 |
| | | | | | Mean | 21 |
| 241 ($S_2$ and $S_3$) | 200 | 228 | 7 | 172 | 133–223 | 24 |
| 262 ($S_2$ and $S_3$) | 170 | 177 | 25 | 132 | 87–173 | 25 |
| 214 ($S_2$ and $S_3$) | 145 | 160 | 26 | 147 | 113–177 | 8 |
| | | | | | Mean | 19 |

TABLE 2.—MEAN FERTILITY INDICES OF 10 SELECTED CLONES IN THE POLYCROSS NURSERY IN 1952 AND MEAN PERFORMANCE FOR FERTILITY INDEX OF THEIR CLONAL AND INBRED PROGENIES IN 1958

| Family | Parental clone | | Inbred progeny | | | Inbreeding depression (%) |
|---|---|---|---|---|---|---|
| | Fertility index (%) | | No. of plants | Fertility index (%) | | |
| | 1952 | 1958 | | Mean | Range | |
| 471 ($S_0$ and $S_1$) | 64 | 79 | 26 | 61 | 38–78 | 23 |
| 383 ($S_0$ and $S_1$) | 45 | 57 | 26 | 45 | 33–66 | 21 |
| 547 ($S_0$ and $S_1$) | 61 | 80 | 26 | 65 | 44–78 | 19 |
| 521 ($S_0$ and $S_1$) | 70 | 78 | 26 | 66 | 46–80 | 15 |
| 613 ($S_0$ and $S_1$) | 66 | 63 | 26 | 46 | 19–76 | 27 |
| 567 ($S_0$ and $S_1$) | 63 | 72 | 26 | 54 | 35–77 | 25 |
| 533 ($S_0$ and $S_1$) | 65 | 81 | 26 | 73 | 45–82 | 10 |
| | | | | | Mean | 20 |
| 241 ($S_2$ and $S_3$) | 52 | 62 | 13 | 44 | 20–73 | 29 |
| 262 ($S_2$ and $S_3$) | 46 | 70 | 26 | 46 | 24–75 | 34 |
| 214 ($S_2$ and $S_3$) | 59 | 78 | 26 | 71 | 39–80 | 9 |
| | | | | | Mean | 24 |

## PERFORMANCE OF INBRED PROGENIES

### *Seed Weight*

Mean data for seed weights of the 10 parental clones and their inbred progenies are presented in a summary form in Table 1. Parental clone means were higher in 1958 than in 1952. There was some evidence of a genotype x year interaction, since relative performance of the clones was not identical in both years. It was noteworthy that in every family the mean of the inbred progeny was below that for the clonal progeny. Data for each family were analysed separately and in every case the mean square for comparison of clonal and inbred progenies was significant at the 1 per cent level. Percentages of inbreeding depression ranged from 6 to 32 per cent and appeared to be positively related to mean seed weights. For the $S_0$–$S_1$ comparisons the mean percentage was comparable to that for the $S_2$–$S_3$ comparisons.

Differences among $S_1$ or $S_3$ plants within each family were significant at the 1 per cent level also. This would indicate segregation for genes conditioning seed weight. Among the 230 inbred plants evaluated, the range in mean seed weight was 87 to 223 milligrams per 50 seeds, or 0.52 to 1.34 grams per 300 seeds. This range is only slightly greater than that for the original 71 clones in the polycross nursery. In four of the 10 families, the highest inbred segregate equalled or exceeded its respective clonal progeny in mean seed weight but none exceeded the highest $S_0$ clone, No. 471. Thus, inbreeding appears to offer little opportunity for isolating genotypes of superior seed weight.

In the analyses of variance, C.V.'s ranged from 2.7 to 4.8 per cent, indicating that two samples of 50 seeds were sufficient for evaluation of these genotypes.

TABLE 3.—MEAN PERFORMANCE FOR SEED YIELD PER PLANT OF THE CLONAL AND INBRED PROGENIES OF 10 SELECTED CLONAL FAMILIES IN 1958

| Family | Parental clone gm./plant | Inbred progeny | | | Inbreeding depression (%) |
|---|---|---|---|---|---|
| | | No. of plants | Gm./plant | | |
| | | | Mean | Range | |
| 471 ($S_0$ and $S_1$) | 50 | 26 | 26 | 6-53 | 49 |
| 383 ($S_0$ and $S_1$) | 38 | 26 | 20 | 7-47 | 47 |
| 547 ($S_0$ and $S_1$) | 126 | 26 | 35 | 7-60 | 72 |
| 521 ($S_0$ and $S_1$) | 98 | 26 | 49 | 9-98 | 50 |
| 613 ($S_0$ and $S_1$) | 70 | 26 | 34 | 20-74 | 51 |
| 567 ($S_0$ and $S_1$) | 96 | 26 | 49 | 17-162 | 49 |
| 533 ($S_0$ and $S_1$) | 106 | 26 | 53 | 15-98 | 50 |
| | | | | Mean | 53 |
| 241 ($S_2$ and $S_3$) | 33 | 14 | 11 | 1-75 | 67 |
| 262 ($S_2$ and $S_3$) | 47 | 26 | 14 | 2-49 | 69 |
| 214 ($S_2$ and $S_3$) | 75 | 26 | 28 | 3-73 | 63 |
| | | | | Mean | 66 |

*Fertility*

Mean results for fertility indices, summarized in Table 2, followed the same general trends as those for seed weight. Clonal means in 1958 were generally higher than those in 1952 and differences in relative performance between years were indicative of a genotype x year interaction. Again, in every family the mean of the inbred progeny was below that of the clonal progeny. This difference was found to be significant at the 1 per cent level in nine families and at the 5 per cent level in Family 383 by analyses of variance. Percentages of inbreeding depression expressed in the $S_3$ generation were comparable to those expressed in the $S_1$ generation, but these reductions did not appear to be related to parent clone fertility.

Differences among inbred plants were found to be significant (1 per cent level) in every family indicating genetic segregation for fertility as evaluated by this index. Among the 247 segregates evaluated the range in fertility indices was 19 to 82 per cent, a slightly greater range than that found in the polycross nursery. In every family, the highest mean fertility index among the inbred segregates was essentially equal to or greater than that for the clonal progeny, but the increase was of appreciable magnitude in five families only. Compared with the best parental clones, inbred segregates offered little promise as source materials for selection to improve fertility.

*Seed Yield*

As was the case with seed weights and fertility indices, mean seed yields of the inbred progenies were below those of the clonal progenies (Table 3). In the analyses of variance, this difference was significant at the 1 per cent level in every family. Percentages of inbreeding depression ranged from 47 to 72 per cent, being considerably larger than those for the two traits previously discussed. The mean percentage of yield reduction was generally greater in the $S_3$ generation than in the $S_1$ generation when compared to respective parental generations. Since seed weight and fertility index are components of seed yield and both exhibited substantial inbreeding depression, a greater depression would be expected for seed

TABLE 4.—PER CENT GENOTYPIC VARIANCE WITHIN INBRED PROGENIES AND PROGENY-PARENT ASSOCIATIONS FOR FOUR AGRONOMIC TRAITS IN 10 SELECTED FAMILIES

| Character | Genotypic variance (%) | | Progeny-parent association | |
|---|---|---|---|---|
| | Range | Pooled | Correlation[1] | Regression[1] |
| Seed weight | 61-91 | 84 | 0.83 | 0.39 |
| Fertility index | 44-94 | 76 | 0.90 | 1.19 |
| Seed yield | 60-92 | 84 | 0.83 | 0.39 |
| Bloom date | 0-66 | 25 | 0.94 | 0.78 |

[1]All values significant at the 1% level with 8 d.f.

yield. However, the difference in relative performance of the $S_1$ and $S_3$ generations cannot be explained on the basis of data obtained in this investigation.

The mean square for testing differences among inbred segregates was significant at the 1 per cent level in every family. Ranges in mean seed yield within inbred progenies were greater in relative magnitude than those for seed weight and fertility (Tables 1 and 2). In eight families, the highest yielding inbred segregate equalled or exceeded the mean performance of the clonal progeny. Moreover, the highest yielding entry in the experiment was an inbred segregate in Family 567.

C.V.'s in these analyses ranged from 14.8 to 68.0 per cent. In the latter case, involving Family 241, only 14 of the 26 segregates produced seed. These C.V.'s for seed yield were higher in every family than those for seed weight or fertility index indicating that more replications or more plants per replication definitely would be desirable in determining seed yield capacity of individual genotypes.

*Bloom Date*

Generally, the mean bloom date for the inbred progeny was one to two days later than mean bloom date for the clonal progeny in the same family. However, this difference was significant in two families only. Significant differences in bloom date among inbred segregates were found in seven families. Among all inbred segregates the range in bloom date was 11 days while the range among the clonal progenies was 8 days. Among the latter, the first to bloom was the clonal progeny of Family 241 and none of the inbred segregates bloomed earlier although a few did bloom on the same date. Thus, inbreeding in this material increased the range in performance but only created opportunities for selection of later blooming types.

*Genotypic Variance and Heritabilities*

Per cent genotypic variance among inbred segregates was estimated for each of the four traits on a within-family basis. The range among families as well as pooled estimates are presented in Table 4. For seed weight, fertility and seed yield, percentages were relatively high indicating large genotypic differences. Pooled estimates for these three traits were quite similar. For bloom date, the percentages were lower in every family. Weather variations during the blooming period affected date of bloom, increasing the environmental effect. This may explain, in part, the lower estimates for bloom date. However, since these estimates would be influenced by the extent of genetic segregation, a possible interpretation is that bloom date is determined by fewer genes or that the parental clones were more homozygous for these genes.

Progeny-parent correlation and regression coefficients also are presented in Table 4. All values were positive and significant at the 1 per cent level. The correlations were of a similar magnitude. For fertility index and bloom date, the regression coefficients were larger than those for seed weight or seed yield, indicating a greater degree of heritability. Progeny-parent associations for seed weight and seed yield were identical whether

TABLE 5.—PHENOTYPIC ($r_P$) AND ENVIRONMENTAL ($r_E$) CORRELATIONS AMONG AGRONOMIC TRAITS IN THE INBRED PROGENIES OF 10 SELECTED CLONES

| Characters correlated | $r_P$ | | | $r_E$ | | |
|---|---|---|---|---|---|---|
| | Range | Pooled | No. significant (5% level) | Range | Pooled | No. significant (5% level) |
| | | | Simple | | | |
| Seed weight and: | | | | | | |
| Fertility | 0.33 to 0.96 | 0.58** | 9 | −0.02 to 0.54 | 0.28** | 5 |
| Seed yield | 0.36 to 0.91 | 0.51** | 5 | −0.30 to 0.19 | 0.00– | 1 |
| Bloom date | −0.53 to 0.04 | −0.26** | 3 | −0.17 to 0.19 | 0.03 | 0 |
| Fertility and: | | | | | | |
| Seed yield | 0.52 to 0.83 | 0.65** | 10 | −0.03 to 0.51 | 0.20** | 4 |
| Bloom date | −0.61 to 0.30 | −0.14* | 4 | −0.35 to 0.21 | −0.01 | 3 |
| Seed yield and: | | | | | | |
| Bloom date | −0.65 to 0.26 | −0.26** | 3 | −0.47 to 0.15 | −0.08 | 2 |
| | | | Partial | | | |
| Seed weight and: | | | | | | |
| Fertility | 0.11 to 0.94 | 0.38** | 6 | 0.00 to 0.59 | 0.28** | 5 |
| Seed yield | −0.30 to 0.86 | 0.22** | 4 | −0.42 to 0.20 | −0.08 | 2 |
| Fertility and: | | | | | | |
| Seed yield | −0.66 to 0.77 | 0.50** | 7 | −0.08 to 0.57 | 0.21** | 4 |

*Significant at the 5 per cent level
**Significant at the 1 per cent level

measured by correlation or regression values. Since seed weight is a component of seed yield, a higher degree of association would be expected for the former trait.

*Interrelationships*

Phenotypic, genotypic and environmental associations among the traits, seed weight, fertility, seed yield, and bloom date, were estimated by covariance analyses. For phenotypic and environmental correlations, the range among individual families as well as pooled estimates are presented in Table 5. Generally, genotypic correlations were quite similar to the corresponding phenotypic values and so are not included in this table. In addition, the number of families in which the respective correlation was significant (5 per cent level) is indicated in the same table. Environmental correlations were generally smaller in magnitude than the corresponding phenotypic correlations and were inconsistent in direction.

All phenotypic simple correlations among seed weight, fertility index and seed yield were positive. Between seed weight and fertility, these were significant in nine of the ten families. Both the pooled phenotypic and pooled environmental correlations were significant at the 1 per cent level. Phenotypic simple correlations between seed weight and seed yield were significant in five families, and the pooled estimate was also significant (1 per cent level). There was no evidence of a consistent environmental association between these two traits, since the pooled correlation was 0.00—. Phenotypic correlations between fertility and seed yield were consistently high and significant, while the pooled environmental correlation was positive and significant at the 1 per cent level.

Correlations involving bloom date were both positive and negative and generally low in magnitude. Pooled phenotypic correlations between seed weight and bloom date and between seed yield and bloom date were negative and significant (1 per cent level), while that between fertility and bloom date was also negative but significant at the 5 per cent level. There was no consistent environmental association between bloom date and any of the other three traits. These results for bloom date indicate that selection for later types would tend to have an adverse effect on seed attributes.

Partial correlations among seed weight, fertility index and seed yield were estimated, using the coefficients of the appropriate simple correlations. The results, also presented in Table 5, are essentially the same as those obtained with simple correlations. All pooled phenotypic partial correlations were positive and significant (1 per cent level). Phenotypic associations between fertility and seed yield were generally higher than between seed weight and seed yield. Since this result was also evident from the simple correlation, it would appear that fertility is more important in the determination of final yield than seed weight. Pooled correlations among seed weight, fertility index and seed yield, indicate no adverse effects of selection for any one of these traits.

## DISCUSSION

The value of inbreeding as a method of forage crop improvement is still controversial. In the perennial forage species, a reduction or loss in performance for many traits almost invariably accompanies inbreeding. In this material, substantial inbreeding depression was noted for seed weight, fertility and seed yield. For seed weight, the percentage of depression appeared to be proportional to the seed weight of the parental clone. The yield depressions were comparable to those which have been observed in corn. These data were, of course, collected from spaced plants overseeded with alfalfa. Further study of these attributes and their interrelationships under solid plantings would be very desirable.

Inbreeding is used by some breeders as a method of isolating superior genotypes or to increase the range in performance. For seed weight, the range among the 71 parental clones was 0.71 to 1.30 grams per 300 seeds. Among the 230 inbred segregates the range was 0.52 to 1.34 grams. Thus, the opportunity for selection of genotypes superior in seed weight was increased only slightly. Despite the fact that seed yield exhibited a greater average per cent inbreeding depression, a number of superior genotypes were isolated. If more parental clones had been used in this study, a greater range in performance among the inbred progenies might have been obtained.

Over 10 years ago, Elliott and Love (2) suggested that, due to irregular chromosome behaviour during meiosis in bromegrass, random sampling of the potential gamete population might not be obtained. In this material, significant differences in performance among inbred segregates within the progenies are indicative of genetic segregation. However, the range in performance may have been reduced from that possible due to irregular meiosis. The clones used in this study have not been examined cytologically. This would appear to be a worthwhile project, since more information is needed on the relationship between cytological behaviour and breeding behaviour.

Considering the difficulties of obtaining selfed seed in bromegrass and the lack of vigour in the inbred plants, inbreeding does not appear to be a desirable procedure for improvement of seed or fertility attributes. In this regard, recurrent selection appeared to be more useful and the results of this phase of the study will be reported at a later date.

Correlations among seed weight, fertility and seed yield indicate that simultaneous improvements of these three traits may be possible. Considering any one correlation, the magnitude of the coefficient varied considerably from family to family. Such variation emphasizes the need for caution in formulating general conclusions from correlation studies. Genotypes, method of planting and season of evaluation can be expected to influence the degree of relationship between characters.

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# CROWN RUST RESISTANT DERIVATIVES FROM CROSSES BETWEEN AUTOTETRAPLOID *AVENA STRIGOSA* AND *A. SATIVA*[1]

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## ABSTRACT

Hexaploid plants having resistance to crown rust were obtained from $F_4$ and $F_5$ progenies of crosses between an autotetraploid of *A. strigosa*, variety C.D. 3820, and two varieties of *A. sativa*, Abegweit and Victory. Seedlings, resistant to races 264, 274, 290 and other races of crown rust, were obtained from segregating populations. The resistant hexaploid derivatives resemble *A. sativa* in most characteristics and can be crossed readily to common oat varieties to give fertile and resistant progeny. It is thus established that genes from diploid species can be utilized for the improvement of common oats.

## INTRODUCTION

Oat breeders are continually faced with the problem of locating sources of resistance to new races of rust. New, highly virulent races of crown and stem rust, capable of parasitizing all commonly grown oat varieties, have been reported during the past 2 years (3, 7). Resistance to crown rust, stem rust and other diseases occurs among varieties of *A. strigosa* and other diploid species (1, 2, 3, 4, 6). One of the main causes of failure to transfer genes from the diploid species to common oats is the incompatibility between varieties of the two groups.

Considerable success has been achieved in overcoming the problem of incompatibility between the two groups through the use of tetraploid and hexaploid forms, compatible with *A. sativa*, as parents at an intermediate stage. Zillinsky (8) obtained hybrid plants from crosses between *A. sativa* ($2n = 42$) and the artificially-induced amphiploid ($2n = 42$) of tetraploid *A. abyssinica* x diploid *A. strigosa*. Tetraploid forms, having genes from diploid species, can also be used as parents in crosses to hexaploid varieties. These might be (a) tetraploids derived from triploid hybrids of crosses between tetraploid and diploid species, or (b) autotetraploids of diploid species. Fertile hexaploid progenies, compatible with *A. sativa*, have been obtained by the authors from crosses involving each of these tetraploids. Five generations of selfing and random outcrossing were required to obtain fully fertile hexaploids from a cross between an autotetraploid of *A. strigosa* from Japan[2] and *A. sativa* (9).

If an intermediate form is used to transfer genes from diploid to hexaploid species, it is necessary to select and test for the presence of desired genes from the diploid parent. Zillinsky *et al.* (10) reported that fertile tetraploid derivatives with genes for resistance to crown and stem rust from *A. strigosa* were obtained from crosses between *A. abyssinica* and *A. strigosa*. The stem rust reaction of such derivatives was reported by Green (3). Sadanaga and Simons (5) were able to transfer the *strigosa*

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[2]This autotetraploid of *A. strigosa* was obtained from I. Nishiyama, Kyoto University, Japan.

gene for resistance to crown rust to hexaploid oats by crossing the resistant derived tetraploid to *A. sativa*, and then backcrossing the pentaploid hybrid to the *A. sativa* parent.

This paper deals with the part of the interspecific hybridization program involving the use of autotetraploids of *A. strigosa* in the transfer of a gene or genes for crown rust resistance to common oats. This project was started at Ames, Iowa, in 1953 and has continued at Ottawa from 1954 to the present time.

## MATERIALS AND METHODS

The variety C.D. 3820 of *A. strigosa* was used primarily because of its resistance to important races of crown and stem rust which parasitize common oats (3, 7). It is grey seeded and is similar in plant type and disease reaction to the variety Saia of the same species. Victory and Abegwait are varieties of *A. sativa* adapted to Canadian conditions but lacking the desired resistance to rusts. Artificially-induced autotetraploids of C.D. 3820 were produced by inverting small vials containing 0.05 per cent aqueous colchicine solution over decapitated tillers of young plants. Two of these autotetraploids, C-2-9 and C-2-10, were used in crosses to Victory and Abegweit. Thirteen hybrid plants were produced from 276 cross pollinations. Viable seeds were obtained from crosses in which the autotetraploid was used as the female parent but not from the reciprocal crosses. The $F_1$ hybrid plants were grown in the field in 1955. All plants were tall, vigorous, late, and tillered profusely. Successive generations were grown in the field in 1956, 1957 and 1958.

Some panicles on each of the $F_1$ plants were bagged to prevent outcrossing to neighbouring plants, others were left unprotected. A brown seeded strain from a cross Same* x Rodney was used in border plots to detect outcrossing from *A. sativa* pollen. Attempts were made to backcross $F_1$ and $F_2$ plants to both *A. sativa* and the autotetraploid parent.

No attempt was made to determine the crown rust reaction of progenies of these crosses in the early generations because of the danger of losing the plants under greenhouse conditions. Tests for reaction to specific races of crown rust were started on $F_4$ and $F_5$ progenies in the fall of 1958. These were made to determine if any of the progenies possessed the same gene or genes conditioning resistance to crown rust as the *A. strigosa* parent. Race 264 of crown rust is a key race used to distinguish resistance possessed by the variety C.D. 3820 from resistance derived from any varieties of *A. sativa*. Strains from $F_5$ and $F_6$ progenies of two crosses were also included in 1958-59 in the U.S.D.A. nursery for virulent races at Puerto Rico for reaction to crown rust race 264.

Reciprocal crosses were made between hexaploid plants resistant to race 264 of crown rust from progenies of each of the crosses 542, 544, and 547 and varieties of *A. sativa* to determine if they were compatible with common oats and also if resistant segregates could be obtained from these crosses.

*"Same" is a tall dark-brown-seeded variety introduced from Sweden.

TABLE 1. — THE HISTORY OF THREE GENERATIONS OF PROGENIES FROM CROSSES BETWEEN AUTOTETRAPLOID *A. STRIGOSA* AND TWO VARIETIES OF *A. SATIVA*

| Cross No. | Identity | 1955 | | | 1956 | | | 1957 | |
|---|---|---|---|---|---|---|---|---|---|
| | | No. of $F_1$ plants | | No. of seeds obtained | No. of $F_2$ plants | | No. of seeds obtained | No. of $F_3$ plants | |
| | | Grown | Prod. seed | | Grown | Prod. seed | | Grown | Prod. seed |
| 542 | C-2-9 x Abegweit | 3 | 1 | 3 | 3 | 3 | 32 | 28 | 19 |
| 543 | C-2-9 x Abegweit | 2 | 2 | 4 | 3 | 1 | 1 | 0 | — |
| 544 | C-2-9 x Victory | 1 | 1 | 4 | 4 | 4 | 31 | 22 | 10 |
| 546 | C-2-9 x Victory | 1 | 1 | 4 | 4 | 1 | 2 | 0 | — |
| 547 | C-2-10 x Victory | 4 | 1 | 3 | 3 | 2 | 7 | 6 | 6 |
| 548 | C-2-10 x Victory | 2 | 2 | 5 | 4 | 1 | 3 | 3 | 0 |

## RESULTS

No seed was obtained from the bagged panicles of $F_1$ or $F_2$ plants, nor from the backcrossed florets of plants from the same two generations. Seeds were obtained from unprotected panicles on some of the plants from $F_1$ and each of the succeeding generations. Dark brown seeds were obtained from six of the twelve $F_2$ plants producing seed in 1956. This would indicate that the degree of outcrossing was high. White seeded $F_2$ plants may also have resulted from outcrossing as white seeded varieties of *A. sativa* were grown in neighbouring plots.

The amount of seed produced by $F_3$ plants in 1957 varied from one or two seeds in some plants to a maximum of 10 grams in others (Table 1). The fertility of the $F_4$ and $F_5$ plants was markedly improved over previous generations with some plants approaching normal fertility. The symbols $F_2$, $F_3$, etc., are used to indicate the generation following the original cross and may not be strictly correct due to outcrossing.

Considerable variability in morphological characters existed among plants of all progenies and in all generations following the $F_1$. Characteristics exhibiting a high degree of variation were vigour, height of plants, number of tillers, panicle type, and seed size. Fatuoids occurred frequently and were found in each of the three most prolific crosses.

Chromosome counts were made on the root tips of some seedlings from $F_3$ and $F_4$ progenies. Considerable variation in chromosome number was observed, ranging from 37 to 42 chromosomes. The chromosome

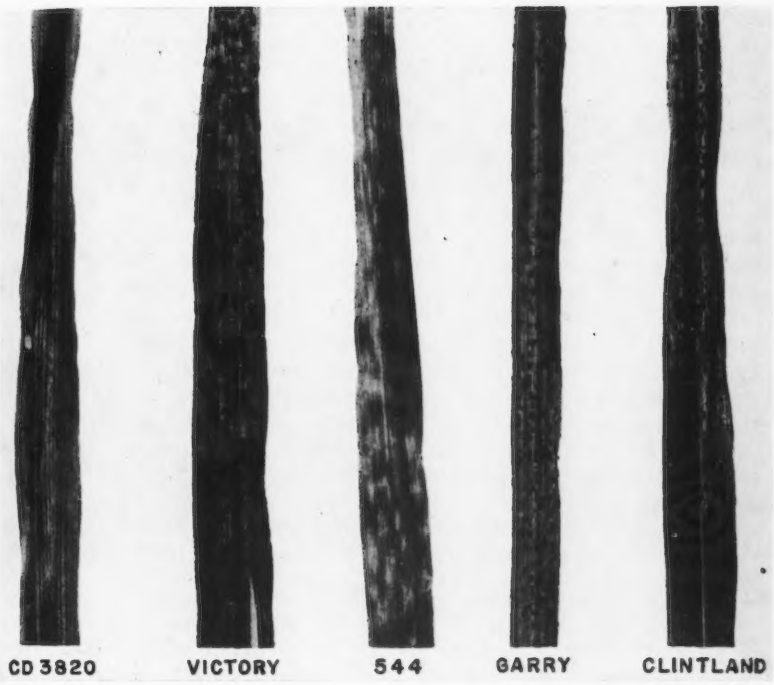


FIGURE 1. Reaction of the parental varieties C.D. 3820 and Victory, a resistant strain from cross 544 (C-2-9 x Victory), and two check varieties Garry and Clintland, inoculated with race 264 of crown rust.

number of the more fertile plants approached 2n = 42. However, some plants having 42 chromosomes were only partially fertile. Resistance to race 264 of crown rust was found among selected strains from each of the three crosses 542, 544 and 547, tested at the seedling stage in the greenhouse at Ottawa (Figure 1.). Several plants having resistance to race 264 from each of the three crosses were inoculated with races 274 and 290 crown rust. All were found resistant to these races. A composite of crown rust inoculum collected from Garry in the field in 1958 was used to inoculate a few plants resistant to the three races of crown rust and again all plants were resistant.

Crown rust reactions to race 264 obtained from the Puerto Rico nursery showed that five of ten strains from cross 542 (C-2-9 x Abegweit) and four of ten strains from cross 547 (C-2-10 x Victory) were resistant to this race.

Attempts at hybridizing plants from $F_4$ and $F_5$ generations of crosses 542 and 547 with varieties of *A. sativa* were more successful than were similar attempts in earlier generations. Hybrid seeds were obtained from reciprocal crosses between plants of 547 and *A. sativa*, and also of 542 and *A. sativa*. Seed set in these crosses was about one-half of that expected in varietal crosses of *A. sativa* and was as high in crosses in which *A. sativa* was used as the female parent as it was when used as a male parent. Differences were observed in amounts of seed produced by the $F_1$ plants from these crosses. Some of this variability may be attributed to unfavourable conditions in the greenhouse and also to an unstable chromosome condition in some of the $F_1$ plants. Segregation for reaction to race 264 was observed among $F_2$ seedlings from these crosses inoculated in the greenhouse in 1959.

## DISCUSSION

Failure to obtain seed from bagged panicles of $F_1$ and $F_2$ hybrids between the autotetraploid of C.D. 3820 and two varieties of *A. sativa* indicates that hybrid pollen is relatively ineffective in fertilization. The seed set among unprotected panicles of $F_1$ and $F_2$ plants averaged between 1 and 2 seeds per plant or about 0.3 to 0.5 per cent. With this amount of sterility existing among the hybrids, the failure to obtain seeds by backcrossing was not unexpected. Since almost one-half of the seeds obtained from $F_1$ plants resulted from outcrossing to border plots of a brown seeded strain of *A. sativa*, it is expected that many or all of the remaining seeds also resulted from outcrossing.

The high incidence of outcrossing makes the determination of the origin of various characteristics exhibited by the progeny difficult. The resistance to crown rust among $F_4$ and $F_5$ progeny in the three crosses 542, 544, and 547 could conceivably have come from varieties in neighbouring plots although no known hexaploid variety resistant in the seedling stage to race 264 has been grown in the nurseries at Ottawa. Even if such a variety existed the probability of outcrossing to progenies of all three crosses is extremely low. The strain from Same x Rodney, used as an outcross check, was not resistant to any of the three crown rust races 264,

274 or 290. The incidence of resistance to race 264 is no higher among progenies from brown seeded $F_2$ plants than among similar progenies from white seeded plants. It is thus assumed that the resistance to crown rust occurring among progenies of the three crosses is conditioned by the same gene or genes that condition resistance in the variety C.D. 3820 (*A. strigosa*).

The results of hybridization between resistant progenies of crosses 542, 544 and 547 and varieties *A. sativa* indicate that such hybrids are readily obtained and that they produce fertile resistant progeny. No difficulty is expected in maintaining the high degree of crown rust resistance in the succeeding generations or from further crosses to varieties of *A. sativa*. This transfer of a gene or genes for resistance to crown rust from a variety of *A. strigosa* to *A. sativa* establishes as a reality the possibility of utilizing genes from the diploid species for the improvement of common oats.

## ACKNOWLEDGEMENTS

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# EFFECT OF SPEAR LENGTH, SPEAR PORTION AND HARVEST DATE ON THE COMPOSITION AND QUALITY OF ASPARAGUS[1]

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## ABSTRACT

Variations in moisture, soluble solids, ascorbic acid and fibre contents of asparagus as affected by spear length, spear portion and harvest date were observed, together with variations in the colour, texture and flavour of the frozen and cooked products.

Highly significant differences in all measurements occurred between the tip, centre and butt of the spear regardless of harvested spear length. The length of harvested spear gave differences in the moisture, soluble solids and fibre content but not the ascorbic acid level. Colour and texture ratings of the frozen and cooked product varied according to spear length while no difference occurred in flavour rating.

The moisture, soluble solids, and ascorbic acid content were influenced by harvest date and there was indication that these were associated with variations in prevailing weather at the time of harvest.

## INTRODUCTION

The past 10 years have been marked by a rapid development in the growing and processing of vegetable crops in southern Manitoba. Asparagus has lagged somewhat in this development because of the time required to establish commercial plantings. However, a moderate acreage has been planted and will be coming into commercial production by 1960. The present study was conducted to assess the variations in composition and quality of the local product, particularly as these variations concerned the spear length and spear portion.

## MATERIALS AND METHODS

A 300-foot row of well-established Registered Washington asparagus was used for the study. The asparagus was harvested in season in 1956 and 1957, by cutting the spears with a knife just below ground level. The harvestings were performed at 4- to 6-day intervals, depending on the prevailing air temperature. Four harvestings were taken in 1956 on the following dates: May 18, 22, 28, and June 2. Six harvestings were made in 1957 on May 6, 13, 17, 24, 29 and June 4. All spears of 2 inches or more in length and ½ to ¾ inches diameter were harvested. The product from each harvest date was segregated into four lots according to spear length. These were 2-4 inches; 5-7 inches; 8-10 inches; and over 10 inches.

The spears of more than 10 inches in length were discarded from the experiment as being of non-marketable quality. Spears of the other three groups were washed and cut into approximately three equal lengths, representing the tip, centre, and butt. Each portion of each size group was analysed in duplicate for moisture, soluble solids, ascorbic acid and fibre content according to the procedures indicated below. Samples of each

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were also blanched in water at 210°F. for 4 minutes, cooled rapidly, frozen and stored at 0 to −5°F. These were later cooked and rated subjectively for colour, texture, and flavour.

*Moisture*: The A.O.A.C. vacuum method (1) was applied to a 3-gram aliquot of blended fresh product.

*Soluble Solids*: A refractometer reading was obtained on the liquid portion of the blended fresh product.

*Ascorbic Acid*: The colorimetric procedure described by Ruck (3) was used.

*Fibre*: The fresh product was pressure-cooked for 10 minutes at 240°F., following which the fibre analysis method of Smith and Kramer (4) was applied.

*Subjective Quality Rating*: The frozen samples were cooked to maximum tenderness with a minimum amount of water, then drained, and rated by a panel of four experienced judges while the product was still warm. Each judge rated colour, texture, and flavour separately on each product. Ratings were based on the following scale: 4.0 – *excellent*; 3.5 – *very good*; 3.0 – *good* or *average*; 2.5 – *fair* or *acceptable*; 2.0 – *poor*; 1.5 – *very poor*.

*Data Analyses*: Composition and quality data were subjected to analyses of variance to determine the relative effects of spear length, spear portion and harvest date on the various composition and quality components. Values for soluble solids and ascorbic acid were transformed to a moisture basis of 90.0 per cent prior to statistical analysis. Fibre readings were not transformed, since the differences were apparent on a fresh weight basis. The subjective ratings of the four judges were averaged for each quality factor and the mean values subjected to variance analyses.

## EXPERIMENTAL RESULTS AND DISCUSSION

The results for 1957 showed the same effects as those for 1956 and served to confirm the experimental findings of the former year. Table 1 includes only the results for 1957 in which six harvestings were made in contrast to the four harvestings in 1956.

The moisture content of the short (2-4 inches) spears was significantly lower than that of the longer spears. The tip was substantially lower in moisture than the other portions of the spear and this agrees with the findings of Culpepper and Moon (2). Products from the first two harvestings were lower in moisture content than those of the other four and appeared to reflect the lack of precipitation during the first half of May in 1957.

Soluble solids content was related inversely to harvested spear length. Also, the tip and centre portions gave significantly higher values than the butt.

There was no significant difference in ascorbic acid content between different spear lengths. A very marked gradation occurred from the tip to the butt with the tip having almost twice the concentration of the butt portion. The products of the fourth and sixth harvestings were significantly higher in ascorbic acid than those of the first and third harvestings.

TABLE 1.—EFFECT OF SPEAR LENGTH, SPEAR PORTION, AND HARVEST DATE ON ASPARAGUS COMPOSITION AND QUALITY

| Spear length | Spear portion | Harvest date | Moisture % | Soluble solids % | Ascorbic acid mg./100g. | Fibre % | Subjective ratings for | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | Colour | Texture | Flavour |
| 2–4″ | | | 90.97 | 8.39 | 49.5 | 0.02 | 2.41 | 2.92 | 2.65 |
| 5–7″ | | | 91.50 | 7.83 | 49.9 | 0.12 | 2.67 | 2.73 | 2.72 |
| 8–10″ | | | 91.58 | 7.38 | 49.8 | 0.26 | 2.84 | 2.63 | 2.66 |
| | | F | 7.11** | 13.63** | 0.05 | 8.18** | 36.91** | 7.01** | 0.71 |
| | | L.S.D. (0.05) | 0.34 | 0.41 | — | 0.12 | 0.10 | 0.16 | — |
| | Tip | | 89.64 | 8.00 | 64.8 | 0.00 | 3.33 | 3.38 | 3.23 |
| | Centre | | 92.08 | 8.21 | 48.5 | 0.04 | 2.63 | 2.98 | 2.76 |
| | Butt | | 92.32 | 7.38 | 36.6 | 0.35 | 1.94 | 1.93 | 2.03 |
| | | F | 158.10** | 10.10** | 177.18** | 21.92** | 378.70** | 186.25** | 269.14** |
| | | L.S.D. (0.05) | 0.34 | 0.41 | 3.1 | 0.12 | 0.10 | 0.16 | 0.11 |
| | | May 7 | 90.42 | 7.27 | 41.6 | 0.20 | 2.69 | 2.79 | 2.72 |
| | | " 13 | 90.95 | 7.96 | 50.3 | 0.07 | 2.57 | 2.80 | 2.68 |
| | | " 17 | 91.76 | 8.03 | 48.7 | 0.11 | 2.65 | 2.85 | 2.74 |
| | | " 24 | 91.82 | 8.10 | 54.1 | 0.16 | 2.70 | 2.80 | 2.71 |
| | | " 29 | 91.52 | 7.76 | 50.1 | 0.12 | 2.62 | 2.70 | 2.60 |
| | | June 4 | 91.62 | 8.08 | 53.5 | 0.15 | 2.58 | 2.61 | 2.59 |
| | | F | 11.10** | 2.81* | 8.63** | 0.59 | 1.22 | 1.30 | 1.50 |
| | | L.S.D. (0.05) | 0.48 | 0.58 | 4.4 | — | — | — | — |

*Significant at 5% level
**Significant at 1% level

A higher fibre content was associated with the longer spears and with the butt portion of the spear.

Colour ratings were higher for the longer spears while texture ratings were higher for the shorter spears. Large differences in colour, texture, and flavour ratings occurred as expected between the tip, centre, and butt portions. There were no significant variations in subjective ratings from one harvest date to another.

The data indicate the differences in composition and quality as a result of variations in spear length, spear portion, and harvest date. Major differences occurred between one spear portion and another and showed that the trimming of the spear had much more effect on the final product quality and composition than did the harvested spear length.

Only minor differences in moisture and ascorbic acid occurred from one harvest date to another. These appeared to be influenced by prevailing weather conditions.

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# RESISTANCE OF INBRED LINES AND HYBRIDS OF SUNFLOWERS (*HELIANTHUS ANNUUS* L.) IN A NATURAL EPIDEMIC OF ASTER YELLOWS[1]

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**ABSTRACT**

Resistance to aster yellows has been identified in sunflowers. In 560 inbred lines and single plant progenies, 356 showed no symptoms of the disease under field conditions. The others showed infections ranging up to 100 per cent. Six entries, observed at three points, showed a range of infection from 0 to 86 per cent and eighteen others, at two points, a range from 0 to 73 per cent. The behaviour was consistent at the different locations.

The resistance to aster yellows appeared to be qualitatively inherited. Single cross hybrids between resistant and susceptible lines showed the resistance to be dominant. It occurred in association both with susceptibility and with resistance to rust. Of 15 lines with evidence of resistance to leaf mottle disease, 13 lines also had resistance to aster yellows.

The sunflower (*Helianthus annuus* L.) is one of many hosts of the aster yellows virus. Severin and Freitag (6) appear to have made the first report of the disease on this host, finding it on the common sunflower in the Castro Valley of California. In Manitoba, a sunflower plant with an abnormal sector in the head was noted in a breeding nursery at Altona in 1952. Because of the sectorial nature of the affected portion it was considered to be due to a genetic chimera. In the following season the prevalence of this type of abnormality was too great to be explained by the chimeral hypothesis. Affected plants were drawn to the attention of the junior author who subsequently attributed the condition to the aster yellows virus and described the symptoms (5).

Aster yellows has occurred on sunflowers in Manitoba each season since 1953. In annual disease surveys it was found in 60 to 65 per cent of the sunflower fields in 1953 to 1955 and again in 1957. It was scarce in 1956 and present in only 20 per cent of the fields in 1958. Infections in individual fields were trace to light in most years except 1957, when 35 per cent of the fields had from 10 to 25 per cent of the plants affected by aster yellows. Observations in farm fields indicated that Beacon was the most susceptible variety grown. Sunrise, the male parent of Advance, was next, but S-37-388, the inbred female parent of Advance, was free of aster yellows in farm fields. Traces of the disease were found in fields of Advance and Mennonite.

The relatively high incidence of aster yellows in 1957, and the striking differences in infection among varieties and breeding material of sunflowers provided an opportunity to make ratings of resistance. This paper records observations made in that year.

## MATERIALS AND METHODS

Most of the observations were made on inbred lines or single plant progenies which originated from controlled crosses. The pedigree system

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TABLE 1. — PARENTAGE OF CONTROLLED CROSSES PRODUCING INBRED LINES OF SUNFLOWERS, MORDEN, MANITOBA

| Cross No. | Parents |
|---|---|
| 6B | 952-70 x Sunrise[2] |
| 18 | 952-72 x Sunrise |
| 25BA, 25BD, 26BC | 953-88-1 x S-37[2] |
| 28 | 953-88-1 x Sunrise |
| 31BA | 953-88-1 x CM79[2] |
| 38, 39 | (953-88-1 x CM13) Sunrise |
| 45 | (953-88-1 x CV061) Sunrise |
| 67, 75 | 953-102-1-1-22 x S-37 |
| 69 | 953-102-1-1-22 x Sunrise |
| 89 | 953-102-1-1-41 x Sunrise |

of breeding was employed using self-pollination until the progenies were considered sufficiently uniform for bulking to form an inbred line.

The parents of the controlled crosses which produced inbred lines or single plant progenies observed in this study are given in Table 1. Origin of the parents was as follows:

Sunrise, 952-70 and 952-72 — From Russian introductions.

S-37, CM13, and CM79 — From the variety Mennonite. S-37 is commonly designated S-37-388 but is being called S-37 in this paper for convenience. It and Sunrise are the parents of the Advance hybrid variety (9).

953-88 — Accession number for the natural cross Sunrise x Texas Wild Annual.

CV061 — From the variety Sunrise.

953-102 — Accession number for the natural cross California Oilseed x Texas Wild Annual. This accession and 953-88 have been sources of rust resistance (3).

Other lines observed were obtained from the varieties Mennonite, Jupiter, Saratov, and Sunrise, and from introductions originating in several countries.

The inbred lines and single plant progenies were grown in the breeding nursery at Morden in single rows 36 inches apart. A full stand consisted of 15 plants 18 inches apart in the rows. A few lines were grown in more than one row. The S-37 and Sunrise alternated as checks throughout the nursery in every 20th row. The number of plants showing symptoms of aster yellows was recorded in the period between flowering and maturity.

Thirty-seven inbred lines and the varieties Advance, Beacon and Sunrise were grown at Winkler, Manitoba, primarily for the purpose of observing their reaction to the leaf mottle disease (4). Aster yellows was prevalent in this test. The number of plants affected by it was recorded in the late bud or early bloom stage. At Winnipeg, 28 inbred lines and single plant progenies, being studied for their reactions to rust, were observed in a field plot and the number of plants affected by aster yellows was noted.

Single cross hybrids produced by hand pollination, without emasculation of the female parent, were yield tested at Morden in 1957. These hybrids were prepared from the inbred lines. They were of two major groups, those in which S-37 was the female parent and those in which Sunrise was the male parent. Other combinations were tested in which

TABLE 2.—FREQUENCY DISTRIBUTION OF BULK INBRED LINES AND SINGLE PLANT PROGENIES OF SUNFLOWERS BY PER CENT PLANTS AFFECTED WITH ASTER YELLOWS UNDER FIELD CONDITIONS, MORDEN, MANITOBA, 1957

| Source of line or progeny | Upper class limits for per cent plants affected | | | | | | | | | | | Total population | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0 | 10 | 20 | 30 | 40 | 50 | 60 | 70 | 80 | 90 | 100 | Plants | % Affected |
| Cross 6B | 3 | 2 | 1 | 2 | 1 | 2 | | | | | | 168 | 17.2 |
| Cross 18 | 3 | 1 | | | | | | | | | | 102 | 1.0 |
| Cross 25BA | 3 | | | | | | 1 | | | | | 57 | 12.3 |
| Cross 25BD | 3 | | | | | | | | | | | 45 | 0.0 |
| Cross 26BC | 22 | 3 | 2 | | | | | | | | | 364 | 1.9 |
| Cross 28 | | | | 1 | | | | | | | 1 | 34 | 61.8 |
| Cross 31BA | 5 | 1 | | | | | | | | | | 90 | 1.1 |
| Cross 38 | 3 | 3 | 7 | 3 | 5 | 5 | 5 | 5 | 3 | 5 | 3 | 689 | 45.6 |
| Cross 39 | 4 | 1 | | | | | | 2 | 2 | | | 106 | 36.8 |
| Cross 45 | 8 | 1 | | 2 | 1 | 4 | 2 | 2 | | | | 337 | 29.1 |
| Cross 69 | 4 | 1 | | | | | 1 | | 1 | | | 200 | 15.0 |
| Cross 67 x S-37[4] | 21 | 3 | | | | | | | | | | 328 | 0.9 |
| Cross 67 x S-37[5] | 44 | 1 | | | | | | | | | | 640 | 0.2 |
| Cross 75 x S-37[4] | 35 | 3 | | | | | | | | | | 568 | 0.5 |
| Cross 75 x S-37[5] | 30 | 1 | 1 | | | | | | | | | 485 | 0.6 |
| Cross 69 x Sunrise[5] | 10 | 5 | 10 | 5 | 10 | 5 | 1 | 4 | 2 | 4 | 1 | 943 | 29.7 |
| Cross 89 x Sunrise[5] | 2 | 4 | 1 | 1 | 3 | | | 1 | | | | 193 | 22.3 |
| Jupiter | 19 | 2 | 22 | | | | | | | | | 343 | 2.0 |
| Mennonite | 44 | 6 | 1 | | | 1 | | | | | | 839 | 3.1 |
| Saratov | 30 | 3 | | | | | | | | | | 509 | 0.8 |
| Sunrise | 1 | 1 | | 2 | 2 | 2 | 2 | | | | | 169 | 33.7 |
| Introductions | 62 | 7 | 1 | | 1 | 1 | 1 | | 1 | | | 1125 | 3.8 |
| Totals | 356 | 49 | 46 | 16 | 23 | 20 | 13 | 14 | 9 | 9 | 5 | 560 | |
| % of all lines | 63.6 | 8.8 | 8.2 | 2.9 | 4.1 | 3.6 | 2.3 | 2.5 | 1.6 | 1.6 | 0.9 | | |

neither S-37 nor Sunrise was a parent. The yield tests consisted of six replicates. The plots were single rows, 16 feet long, spaced 3 feet apart. At maturity most plots contained 20 to 30 plants. The number of plants showing symptoms of aster yellows in these trials was recorded shortly before maturity.

## RESULTS

A summary of the incidence of aster yellows in the inbred lines and progenies grown at Morden is given in Table 2. Only entries in which 10 or more plants were available for observation have been included in this

TABLE 3. — PERCENTAGE OF PLANTS AFFECTED BY ASTER YELLOWS UNDER FIELD CONDITIONS IN GROUPS OF RELATED INBRED LINES AT THREE LOCATIONS IN MANITOBA, 1957

| Pedigree | Morden | | Winkler | | Winnipeg | |
|---|---|---|---|---|---|---|
| | No. of plants | % Affected | No. of plants | % Affected | No. of plants | % Affected |
| 25BA-11-2-1-4-2 | 13 | 54 | — | — | — | — |
| 25BA-11-2-1-5-3-1 | 14 | 0 | — | — | — | — |
| 38-2-3-5 | 15 | 93 | 44 | 41 | 16 | 63 |
| 38-2-3-9-1-3 | 15 | 60 | — | — | 14 | 86 |
| 38-2-3-10-3-1 | 15 | 100 | — | — | — | — |
| 38-2-3-23-3 | 13 | 62 | — | — | 13 | 77 |
| 38-4-3-2-1-1 | 15 | 73 | — | — | 17 | 53 |
| 38-20-4-5-1-3 | — | — | 67 | 30 | 14 | 57 |
| 38-20-4-14-2 | 15 | 13 | — | — | — | — |
| 38-20-4-16-2-2 | 14 | 57 | — | — | — | — |
| 38-20-5-1-1-6 | 15 | 0 | — | — | — | — |
| 39-16-2-2-1 | 15 | 73 | 32 | 53 | — | — |
| 39-16-2-2-2-3 | 14 | 64 | 78 | 26 | — | — |
| 39-16-3-5-2 | 12 | 0 | — | — | 13 | 0 |
| 39-27-5-1-4-2 | 13 | 0 | — | — | 12 | 83 |
| 39-27-5-1-5-1 | 14 | 0 | — | — | — | — |
| 45-15-1-6-1-1 | 9 | 67 | — | — | 7 | 29 |
| 45-24-2-2-3-3 | 4 | 25 | — | — | 6 | 17 |
| 45-47-2-2-2-3-1 | 15 | 40 | — | — | 14 | 57 |
| 45-47-2-2-2-3-2 | 13 | 46 | — | — | — | — |
| 45-47-2-2-2-2-1-1 | 14 | 0 | — | — | — | — |
| 45-47-2-2-2-2-1-2 | 12 | 0 | — | — | — | — |
| 45-47-2-2-2-2-1-3 | 11 | 0 | — | — | — | — |
| 45-68-1-4-1-2-2 | 12 | 0 | 65 | 14 | 21 | 0 |
| 69-6-4-2 | — | — | 38 | 0 | 14 | 0 |
| 69-8-1-5 | — | — | 18 | 22 | 4 | 25 |
| 69-17-8-1 | 51 | 0 | — | — | — | — |
| 69-17-1-1-5 | 12 | 75 | — | — | — | — |
| 26BC-40-1-7 | 14 | 0 | 54 | 4 | 22 | 0 |
| 26BC-58-1-1-4 | 13 | 0 | — | — | 13 | 0 |
| 31BA-17-4-4-4 | 14 | 0 | 48 | 0 | 15 | 0 |
| Advance | 958 | 0 | 51 | 4 | — | — |
| S-37 | 319 | 1 | 49 | 0 | 151 | 0 |
| Sunrise | 286 | 20 | 59 | 27 | — | — |
| Beacon | 1545 | 5 | 58 | 14 | — | — |

table. Sixty-four per cent, or 356 of the 560 entries recorded in the table, did not show any symptoms of aster yellows. At the other extreme, 70 lines fell into the classes with upper limits of 50 per cent or over. As shown in Table 3, all 15 plants in one line from cross 38 were affected by this disease so that the range of infection was actually from 0 to 100 per cent.

The resistant lines or those with low infection, and the susceptible lines or those with high infection tended to fall into distinct groups on the basis of origin. In general, crosses in which the S-37 line was a parent produced a high proportion of resistant lines. This was evident in the resistant lines as shown in crosses 25BA, 25BD, 26BC and the crosses 67 and 75 backcrossed recurrently to S-37. The varieties Jupiter, Mennonite and Saratov and the introductions all gave a high proportion of resistant lines. On the other hand the variety Sunrise, crosses in which it was a parent, including 38, 39, 45 and 69, and material in which it was a recurrent parent produced a high proportion of susceptible lines.

Table 2 also shows that there was a higher percentage of plants affected in the total populations of the crosses in which Sunrise was a parent.

Table 3 provides information on some of the lines that were grown at more than one location and also data for groups of related lines that were grown at Morden only. In these pedigrees the first group of digits refers to the cross number as given in Table 1, and subsequent groups of digits or single digits are selection numbers given to single selfed plants in successive generations.

Table 3 shows that the lines behaved consistently when grown at two or more points. No aster yellows appeared on line 31BA-17-4-4-4 at any of the three locations and on S-37 there was none at two locations and a low incidence at Morden. By contrast, the line 38-2-3-5 was severely affected at all three sites. A few anomalies occurred, most striking of which was the high infection percentage on line 39-27-5-1-4-2 at Winnipeg compared with none at Morden. A check on the seed source indicated that the identity of seed of this line used at Winnipeg was questionable, which may explain the discrepancy in behaviour at the two locations.

TABLE 4.—SUMMARY OF REACTION OF SINGLE CROSS SUNFLOWER HYBRIDS TO ASTER YELLOWS UNDER FIELD CONDITIONS, MORDEN, MANITOBA, 1957

| Type of hybrid | No. of hybrids | No. showing affected plants | Range of per cent infection | Total population | |
|---|---|---|---|---|---|
| | | | | Plants | Per cent affected |
| S-37 x resistant | 12 | 0 | 0.0-0.0 | 1719 | 0.0 |
| S-37 x susceptible | 28 | 1 | 0.0-2.9 | 4072 | 0.1 |
| Resistant x resistant | 6 | 0 | 0.0-0.0 | 996 | 0.0 |
| Resistant x susceptible | 13 | 3 | 0.0-3.0 | 2002 | 0.3 |
| Susceptible x resistant | 1 | 1 | — | 148 | 10.1 |
| Susceptible x susceptible | 11 | 11 | 5.5-38.9 | 1687 | 17.8 |
| Resistant x Sunrise | 12 | 2 | 0.0-2.3 | 1892 | 0.2 |
| Beacon | — | — | 2.7-15.6* | 1545 | 4.7 |
| Advance | 1 | 0 | — | 958 | 0.0 |

*In four different tests

Table 3 further shows that resistant and susceptible lines emerged from the same cross. This was most striking in the two lines of 25BA-11-2-1 and in the six lines of 45-47-2-2-2 origin. In these two instances the segregation for resistant and susceptible lines occurred after three and four generations, respectively, of selfing.

Table 4 contains a summary of the data secured from the yield trials of the experimental hybrids. Parental lines designated as resistant had either no plants or a single plant affected by the disease in the nursery, while those designated as susceptible had two or more plants affected or a percentage of infection varying from 13 to 100. It will be recognized from Table 3 that S-37 is a resistant line and that Sunrise is a susceptible line and that Advance thus is a resistant x susceptible combination. Out of 73 hybrids involving a resistant parent, only 7 were affected by aster yellows. Of these 7 the affected plants did not exceed 3 per cent in six hybrids compared with levels up to 39 per cent in susceptible x susceptible combinations. The seventh hybrid was a susceptible x resistant combination in which 10 per cent of the plants were affected. As there were only 45 per cent actual hybrids in this entry, the diseased plants could easily have been self plants of the susceptible female parent.

This resistance to aster yellows has occurred in association both with susceptibility and with resistance to rust. For example, the 202 lines from the varieties Jupiter, Mennonite, Saratov, and the introductions included in Table 2, were all susceptible to rust. Of these, 173 were in the zero aster yellows class or the class with an upper limit of 10 per cent. Of the 333 lines from those crosses involving the 953-88 and 953-102 sources of rust resistance, all of which were selected for resistance to rust, 194 were free of aster yellows. Other sources of rust resistance were reported in an earlier paper (3). Among these, progenies uniformly resistant to rust from the accessions 306, 403-3 and 403-4 were all free of aster yellows or showed a low percentage of plants with symptoms at both Winnipeg and Morden.

There was a different pattern of association between aster yellows resistance and resistance to leaf mottle disease. Five lines which showed low incidence of leaf mottle were also either free of, or slightly affected by aster yellows (4). In 1958 an additional 10 lines sustained low leaf mottle injury. Of these, 8 were free of aster yellows or had not more than 10 per cent of their plants affected by it in 1957. Only 1 of the 10 was severely affected by aster yellows. No information was available on the tenth line. Thus, of a total of 15 lines in two seasons which exhibited evidence of resistance to leaf mottle disease, 13 lines also had resistance to aster yellows. The pedigree of the 13 lines shows that they originated from seven different crosses or varieties. In contrast many lines resistant to aster yellows were not resistant to leaf mottle. The best examples of such lines were S-37 and 31BA-17-4-4-4, both of which showed severe injury by leaf mottle in 1958 but, as is seen in Table 3, were free or nearly free of aster yellows.

## DISCUSSION

Intraspecific differences in reaction to aster yellows among its hosts are rare. Thompson in 1944 (8) reported two strains of *Lactuca serriola* L.

with infections of 7 per cent or less when two other strains in the same tests showed infections of 28 per cent. One strain of *L. saligna* had a mean infection of 7.4 per cent and another 3.7 per cent over a 4-year period in which disease potential was high, as indicated by mean infection for *L. villosa* of 63.2 per cent. Thompson described the strains as having a marked ability to escape infection rather than terming them resistant. Harvey and Schroeder (1) working with 14 varieties and strains of carrots in the field for 3 years, found consistent and highly significant differences in aster yellows infection, ranging from 14.4 to 50.8 per cent. They showed in cage tests that these differences may have been due to varietal preference of the vectors. Thomas (7) demonstrated a differential varietal expression in carnations of bud malformation caused by aster yellows. The behaviour was dependent on high temperature. Lee and Robinson (2) found the Trianon Cos variety of lettuce to be more tolerant to the disease than seven other varieties tested.

The resistance of sunflowers to aster yellows appears to be the most definite yet discovered in any crop plant because many lines were free of symptoms of the disease when many others observed under the same conditions showed infections over 30 per cent with a few ranging up to 100 per cent. The consistency in behaviour at three locations is further evidence that resistance to aster yellows actually exists in sunflowers.

The resistance appears to be widely distributed, particularly in Europe, as the varieties and introductions which gave resistant lines were obtained from Scotland, Holland, Germany, Austria, Hungary, Norway, Finland, Russia, Malta and Turkey.

The nature of the resistance is undetermined at present. It may be due to a true physiological resistance which prevents the virus becoming established in the plants or to a resistance to its insect vector. Greenhouse and laboratory experiments with viruliferous leafhoppers on caged plants at Winnipeg have so far given inconclusive results, especially because of difficulty in making reliable diagnoses of symptoms on seedling sunflowers. It was found, however, that the vectors would feed on yellows resistant S-37 in cages also containing susceptible Sunrise seedlings, suggesting that the disease resistance of S-37 was not based on a varietal preference of the vector.

The emergence of resistant and susceptible lines from crosses after three and four generations of inbreeding is evidence that this resistance to aster yellows in sunflowers is qualitatively inherited and that it may be controlled by a single gene. The observations on the single cross hybrids indicate that the character is dominant.

Determination of the exact nature of the inheritance depends on the development of greenhouse techniques for study of segregating populations, preferably in the seedling stage. The sporadic nature of severe outbreaks prevents effective study of the inheritance of resistance under natural conditions in Manitoba. In only one of the seven seasons since the disease was first observed on sunflowers in the province has the outbreak been severe

enough to differentiate resistant and susceptible material reliably, and then only on a line or progeny basis rather than as individual plants.

The occurrence of 13 lines resistant to aster yellows among 15 lines found in two seasons to be resistant to leaf mottle is a frequency much higher than would have occurred by chance alone. This indication that lines resistant to leaf mottle may also be resistant to aster yellows may be utilized for selecting for resistance even in the absence of severe outbreaks of aster yellows. Severe infections of leaf mottle in infested soil under field conditions are obtainable every season. Growing material in an area of the continent where aster yellows occurs regularly in high frequency is another prospective means for effectively evaluating the reaction of inbred lines to the disease.

From the practical aspect the evidence that resistance to aster yellows is genetically dominant is most encouraging. The single or topcross hybrid Advance (9) has played a major role in Canadian sunflower production and it or similar hybrids appear likely to continue to do so for considerable time. Thus, if the female parent of such hybrids is a line carrying resistance to aster yellows, the resulting hybrids will also be resistant to the disease regardless of the genotype of the male parents with respect to this character. By this means the lines of Sunrise parentage, many of which have high combining ability, or others, even though susceptible to the disease, may be utilized in commercial varieties. Fortunately, the S-37 or female parent of the Advance hybrid, and two other inbred lines which have good potential as female parents in commercial single crosses or topcrosses, possess resistance to aster yellows.

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# ESSAI DE DIFFERENTS PRODUITS CHIMIQUES COMME DESINFECTANTS DES ECLATS (GERMES) DE POMMES DE TERRE INOCULES AVEC *CORYNEBACTERIUM SEPEDONICUM*[1]

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## RESUME

Quarante-sept substances chimiques, comprenant des antibiotiques, des détergents, des composés d'ammonium quaternaire, des sels de mercure, des désinfectants et des protectants, furent utilisés dans la désinfection des éclats de pommes de terre inoculés avec *Corynebacterium sepedonicum* (Spieck. et Kott.) Skapt. et Burkh. A l'exception du bichlorure de mercure, toutes ces substances chimiques furent inefficaces, laissant plus de 25 à 50 pour cent de buttes flétries au moment de la récolte. Le trempage des éclats contaminés dans une solution de bichlorure de mercure acidifié (2:1000 + 1 pour cent HCl) durant 10 minutes a réduit la maladie dans des proportions de 93 à 98 pour cent. Une semence infectée à 1 pour cent et désinfectée de la même façon a produit une récolte apparemment saine.

## INTRODUCTION

L'efficacité du bichlorure de mercure ($HgCl_2$) dans la désinfection des éclats de pommes de terre contre les maladies bactériennes est reconnue depuis longtemps; dès 1909, Morse le recommande contre la jambe noire (7). Ses usages comme désinfectant sont assez nombreux, puisqu'il peut servir à la désinfection du couteau, des instruments, des sacs, et des éclats de pommes de terres (1, 4, 8, 9). Il aurait de plus l'avantage de protéger les éclats contre les agents de pourriture venant du sol, et certaines maladies telles que la rhizoctonie, la gale et la jambe noire (2). Malheureusement, le bichlorure de mercure perd son pouvoir germicide assez rapidement à l'usage (3), et il possède l'inconvénient d'être corrosif et de réduire quelque peu le pouvoir germinatif et les rendements.

La découverte des antibiotiques fit espérer en une panacée capable de réduire au minimum les méfaits de la flétrissure bactérienne. Van Schaak (10) obtint quelques bons résultats avec la streptomycine comme désinfectant des éclats de pommes de terre inoculés avec *C. sepedonicum.* Ses résultats furent contredits par Woolliams[2] qui démontra qu'un trempage de 15 minutes dans une solution de streptomycine à 400 p.p.m. était insuffisant pour détruire les bactéries de la flétrissure de la pomme de terre.

La mise sur le marché de nombreux composés chimiques recommandés comme d'excellents germicides ou agents thérapeutiques, nécessite une expérimentation continuelle afin de trouver un agent de désinfection supérieur au bichlorure de mercure et n'en possédant pas les inconvénients. Depuis quelques années, des études sont poursuivies au Laboratoire des Recherches à Ste-Anne-de-la-Pocatière dans le but de sélectionner un ou plusieurs de ces produits.

## METHODES

Avant leur désinfection, les éclats sont d'abord inoculés en coupant les tubercules avec un couteau fraîchement contaminé et en les plongeant

[1]Contribution du Laboratoire régional des Recherches, Ministère de l'Agriculture du Canada, Ste-Anne-de-la-Pocatière, Qué.

[2]Woolliams, G. E. Report of meetings at Portland, Oregon, Jan. 17-20, 1956. *Lettre personnelle à l'auteur,* 29 février, 1960.

TABLEAU 1. — ESSAI DE DÉTERGENTS, D'ANTIBIOTIQUES ET D'AUTRES PRODUITS CHIMIQUES COMME DÉSINFECTANTS DES ÉCLATS (GERMES) DE POMMES DE TERRE INOCULÉS AVEC *C. SEPEDONICUM* (1955)

| Traitement | Concentration et durée | % Buttes atteintes de flétrissure[1] |
|---|---|---|
| Témoins inoculés | — | 48 à 64 |
| $HgCl_2$ (1:1000) + détergents | 1 c. à soupe / gal. Trempage 15 à 60 min. | *1.0 à 3.2* |
| Détergents (Rinso, Vassol, Tarpol) | 1 à 3 c. à soupe /gal. Trempage 15 à 60 min. | 37 à 39 |
| Streptomycine (Agristrep) | 25 à 50 p.p.m. Trempage 15 à 30 min. | 36 à 51 |
| Captan 75 (Fongicide) | 1.5 à 3.0 lb./100 gal. Trempage 2 à 10 min. | 47 à 75 |

[1]Observation visuelle des symptômes sur les tubercules

TABLEAU 2. — ESSAI DE COMPOSÉS QUATERNAIRES, D'ANTIBIOTIQUES ET D'AUTRES PRODUIT CHIMIQUES COMME DÉSINFECTANTS DES ÉCLATS (GERMES) DE POMMES DE TERRE INOCULÉS AVEC *C. SEPEDONICUM* (1956-1957)

| Traitement | Concentration et durée | % Buttes atteintes de flétrissure[1] | Rendement boisseaux à l'acre[2] |
|---|---|---|---|
| Témoin sain | — | 0 | 410 |
| Témoin inoculé | — | 83 | 129 |
| $HgCl_2$ acidifié | 2:1000 + 1% HCl/10 min. | *4* | 295 |
| $HgCl_2$ | 1:1000/20 min. + Vassol | 16 | 336 |
| $R_3L$ | 1:100/15 min. | 30 | 298 |
| Agrimycine | 200 p.p.m./30 min. | 32 | 258 |
| $R_2L$ | 1:100/5 min. | 41 | 314 |
| Streptomycine | 200 p.p.m./30 min. | 41 | 251 |
| $HgCl_2$ | 1:1000/20 min. | 47 | 250 |
| Lysol | 1% — trempage instantané | 47 | 232 |
| Agrimycine | 100 p.p.m. 3 arrosages | 76 | 191 |
| Agrimycine | 200 p.p.m. 3 arrosages | 77 | 183 |
| Streptomycine | 200 p.p.m. 3 arrosages | 80 | 213 |
| Streptomycine | 100 p.p.m. 3 arrosages | 89 | 142 |

[1]Observation visuelle des symptômes sur les tubercules
[2]d. = 51 à 5%
68 à 1%

dans une suspension bactérienne. Cette méthode d'inoculation permet 80 à 90 pour cent d'infection. Les éclats sont ensuite immergés dans les solutions désinfectantes pour des périodes de temps définies et plantés sans délai. A moins d'indication contraire, la désinfection suivit immédiatement l'inoculation. La quantité d'éclats et le nombre de parcelles (répétitions) furent en général suffisants pour permettre l'analyse statistique.

TABLEAU 3. — EFFICACITÉ DU BICHLORURE DE MERCURE ACIDIFIÉ ET DE LA FORMALINE SEULS OU EN MÉLANGE AVEC DES DÉTERGENTS COMME DÉSINFECTANTS DES ÉCLATS (GERMES) DE POMMES DE TERRE INOCULÉS AVEC *C. SEPEDONICUM* (1958)

| Traitement | % Buttes atteintes de flétrissure[1] | Rendement boisseaux à l'acre[2] |
|---|---|---|
| Témoin sain | 0 | 220 |
| Témoin inoculé | 68 | 112 |
| $HgCl_2$ 2-1000 + 1% HC1/10 min. | *2* | *169* |
| $HgCl_2$ 2:1000 + 1% HC1/10 min. + Alipal 0.5% | 3 | 126 |
| $HgCl_2$ 2:1000 + 1% HC1/10 min. + Cationic 0.5% | 2 | 138 |
| $HgCl_2$ 2:1000 + 1% HC1/10 min. + Triton X-100, 0.5% | 0 | 77 |
| Formaline 10%/5 min. | 13 | 99 |
| Formaline 10%/5 min. + Alipal | 10 | 71 |
| Formaline 10%/5 min. + Cationic | 8 | 72 |
| Formaline 10%/5 min. + Triton X-100 | 8 | 31 |

[1]Observation visuelle des symptômes sur les tubercules
[2]d. = 97 à 1%
72 à 5%

TABLEAU 4. — ESSAI DE PRODUITS CHIMIQUES COMME DÉSINFECTANTS DES ÉCLATS (GERMES) DE POMMES DE TERRE INOCULÉS AVEC *C. SEPEDONICUM* (1958)

| Nom du produit[1] | % Buttes flétries[2] | % Tubercules flétris[3] |
|---|---|---|
| Témoin inoculé | 88.5 | 47.8 |
| $HgCl_2$ acidifié 2:1000 + 1% HC1 | *8.0* | *3.1* |
| Saumure: Solution saturée de NaC1 | 11.1[4] | 2.4 |
| Bouillie bordelaise 5:2.5:100 | 35.9 | 10.9 |
| Oxyde jaune d'Hg 2:1000 | 60.5 | 25.6 |
| Triton X-100 0.5% | 62.1 | 28.3 |
| Ilotycin 1:1000 | 62.9 | 29.1 |
| Hyamine 2389 2-1000 | 63.0 | 27.3 |
| Hyamine 1622 1:1000 | 64.8 | 32.4 |
| Bionol A-50 2:1000 | 65.0 | 27.1 |
| Teramine 5:1000 | 68.7 | 34.8 |
| Katapone VV328 3:1000 | 69.7 | 31.7 |
| Semesan Bel 1 lb./10 gal. | 72.6 | 38.7 |
| Hyamine 10X 1:1000 | 76.1 | 40.4 |
| Arquad S-50 1:1000 | 87.0 | 50.1 |
| Germ-i-tol 1:1000 | 78.2 | 41.7 |
| Dichloran 60% 1:1000 | 77.6 | 37.7 |
| DMBC 75% 1:1000 | 78.8 | 46.4 |
| Cetyl Peridinium Chloride 2:1000 | 75.3 | 33.3 |
| Cetyl Trimethyl $NH_4$ 2:1000 | 91.7 | 59.2 |
| Stearyl Trimethyl 2:1000 | 90.6 | 57.7 |
| H.T. 70% 2:1000 | 89.9 | 52.0 |
| Laurol 1:1000 | 81.6 | 39.4 |
| Aerosol OT-B 2:1000 | 86.5 | 54.0 |
| Purasan AD-50% 2:1000 | 83.7 | 43.7 |
| Purasan DC-50% 1:1000 | 85.0 | 44.6 |
| Hartexsan 1:1000 | 87.2 | 42.9 |
| Roccal 1:1000 | 84.6 | 45.3 |
| Cationic SP 0.5% | 93.8 | 59.9 |
| Alipal CO-436 0.5% | 78.8 | 45.6 |
| BTC-824 1:1000 | 84.1 | 41.7 |
| Tetrosan 60% 2:1000 | 86.2 | 41.7 |

[1]Durée du traitement: 10 minutes
[2]Observation visuelle des symptômes sur le feuillage et[3] sur les tubercules
[4]Le pouvoir germinatif est détruit à 95%

## RESULTATS

Les tableaux 1 et 2 pour les années 1955 et 1956 montrent la supériorité de $HgCl_2$ acidifié sur les antibiotiques tels que la streptomycine et l'agrimycine, sur les détergents tels que le Rinso, le Vassol ou le Tarpol, sur les composés quaternaires tels que le $R_2L$, et sur d'autres désinfectants ou "protectants" tels que le Lysol et le Captan. Le bichlorure de mercure diminue l'incidence de la flétrissure bactérienne de la pomme de terre dans une proportion de 96 à 98 pour cent, et ce résultat peut atteindre 100 pour cent lorsqu'une semence ne contient que 1 pour cent d'infection. Les autres substances utilisées diminuent l'infection de 0 à 70 pour cent avec une moyenne de 48 pour cent. Ces résultats furent les mêmes en appliquant les traitements deux heures après l'inoculation.

Pour permettre une meilleure pénétration des désinfectants dans les tissus, des détergents anioniques tels qu'Alipal, cationiques ou non ioniques tels que Triton X-100 furent mélangés aux solutions de bichlorure de mercure et de la formaline. L'addition de ces détergents (0.5 pour cent) n'eut aucun effet sur le pouvoir germicide de $HgCl_2$ et de la formaline; par ailleurs, ils réduisirent les rendements (Tableau 3).

En 1958, plus de 32 composés chimiques furent utilisés dans la désinfection des éclats inoculés. La plupart de ces composés possèdent un coefficient phénolique élevé et sont reconnus comme de puissants agents de désinfection en technologie médicale (5). Aucun de ces produits ne s'est montré aussi efficace que $HgCl_2$ acidifié qui prévint la maladie dans une proportion de 92 pour cent pour les buttes et de 97 pour cent pour les tubercules (Tableau 4). La bouillie bordelaise se classa deuxième avec 36 pour cent de buttes flétries et 11 pour cent de tubercules atteints de flétrissure. Dans tous les autres traitements, il y avait plus de 40 pour cent de buttes flétries et plus de 25 pour cent des tubercules étaient atteints de pourriture.

En 1959, cinq nouvelles substances furent utilisées dans la désinfection des éclats dont un antibiotique de découverte récente, la Vancomycine, qui posséde un pouvoir germicide assez élevé envers les bactéries pathogènes,

TABLEAU 5. — ESSAI DE PRODUITS CHIMIQUES COMME DÉSINFECTANTS DES ÉCLATS DE POMMES DE TERRE INOCULÉS AVEC *C. SEPEDONICUM* (1959)

| Traitement | Concentration et durée | % Buttes flétries[1] |
|---|---|---|
| Témoin inoculé | | 75[2] |
| $HgCl_2$ acidifié | 2:1000 + 1% HCl/10 min. | 2 |
| Vancomycine | 200 p.p.m./40 min. | 24 |
| Emcol E-11 | 2:1000/10 min. | 33 |
| Ioclide | 100 p.p.m./20 min. | 59 |
| Chlorure de sodium | 5%/30 min. | 59 |
| Alrodyne 8020 | 5:1000/10 min. | 63 |
| Emulsept 2X | 2:1000/10 min. | 70 |
| Vancomycine | 50 p.p.m. 2 arrosages | 72 |

[1]Observation visuelle des symptômes sur tubercules
[2]Moyenne des expériences sur sol lourd argileux et sur sol léger sablonneux

surtout celles du genre *Corynebacterium* (6). Aucune de ces substances ne s'est montrée aussi efficace que $HgCl_2$ acidifié dont l'action désinfectante ne laissa que 2 pour cent de buttes malades, alors que la Vancomycine permit une infection de 24 pour cent. L'efficacité de $HgCl_2$ fut encore augmentée par l'agitation des éclats contaminés dans une baratte durant la désinfection. Malheureusement, ce traitement affecta gravement le pouvoir germinatif des éclats.

Bien que le bichlorure de mercure diminue généralement le pourcentage de germination et réduise les rendements, cette diminution et cette réduction ne sont pas toujours significatives.

En résumé, le bichlorure de mercure, malgré de graves inconvénients, demeure encore le meilleur désinfectant pour les éclats (germes) de pommes de terre atteints de flétrissure bactérienne.

## REMERCIEMENTS

Nous remercions nos collègues et les autres membres du personnel du laboratoire qui ont apporté généreusement leur concours dans l'exécution de ces travaux.

## SUMMARY

Forty-seven chemicals, including antibiotics, detergents, quaternary ammonium salts, mercury compounds, disinfectants and protectants, were tested for their effectiveness as potato seed piece disinfectants after inoculating each potato set with bacterial ring rot. None of the substances tested was as effective as a solution of acidified mercuric chloride (2:1000 + 1 per cent HCl) in a 10-minute dip treatment which gave 93 to 98 per cent control. A more effective control of the disease (100 per cent) was obtained when seed stock contained only 1 per cent diseased tubers.

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# THE EFFECT OF EMBRYO TRANSPLANTATION ON SEED SET IN SOME WHEAT-RYE CROSSES[1]

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## ABSTRACT

Experiments were conducted to give information on the methodology and value of embryo transplantation in wheat-rye crosses. Embryos of diploid, tetraploid and hexaploid wheats were transplanted to wheat, rye, and alien endosperm types. Plants derived from transplanted embryos were used for crosses with *Secale cereale*. A modified method for transplanting embryos to another endosperm was developed. Results of test crosses indicate that crossing results could not be improved by transplanting embryos to different endosperm types. Findings support the theory of the presence of "crossability genes" in some wheat parents which appear to be the same in tetraploid as in hexaploid wheats.

## INTRODUCTION

Interspecific and intergeneric crosses are of special interest to plant breeders because of their potential value in developing new crop species. However, total or partial sterility occurs in most of these crosses. Methods are needed which would increase the frequency of obtaining viable seeds from such crosses. Many investigations have been made in this field especially with cultivated plants such as potatoes, beets and cereals (3, 4, 6, 15, 18). Previous reports dealing with hybridization experiments between wheat and rye (7, 8, 9, 16) concluded that the success of crosses between these two genera depended upon the presence of crossability genes in the wheat parent, regardless of whether wheat was used as the female or the male parent. Other reports (4, 5, 11, 12) suggested that this so-called "crossability-barrier" could be overcome by embryo transplantation. Embryos of one species were transferred to the endosperms of another and plants derived from these reconstructed seeds were used as the female in crosses with plants of the donor endosperm type. Some workers (4, 11) indicated that this embryo transplantation improved seed set in some of their interspecific and intergeneric crosses.

An experiment was designed to yield additional information on the methodology and value of embryo transplantation in wheat-rye crosses. It was carried out at the University of Manitoba during the summers of 1957 and 1958.

## MATERIALS AND METHODS

In all crosses between wheat and rye, wheat was the female parent and the Canadian spring rye, *Secale cereale* (Prolific), was the male parent. Diploid *Triticum monococcum* (Einkorn), tetraploid *Triticum durum* (Stewart) and hexaploid *Triticum vulgare* (Prelude) were included in crosses in both years. Tetraploid *Triticum Timopheevi* and hexaploid *Triticum vulgare* (Kenya Farmer) were used in 1957 only.

As proposed by Pissarev and Vinogradova (11, 12) embryos from the selected wheat species and varieties were transplanted to the endosperm of the "Prolific" rye male parent. Following the suggestions made by Hall

[1]Contribution from the Division of Plant Science, University of Manitoba, Winnipeg, Man.

TABLE 1. — SEED SET IN CROSSES OF TRANSPLANTED DIPLOID, TETRAPLOID AND HEXAPLOID WHEATS WITH *S. CEREALE* (PROLIFIC) IN 1957

| Female Parent | Apparent Seed Set | | | |
|---|---|---|---|---|
| | Viable seeds | | Inviable seeds | Seeds |
| | Normal | Aborted | | |
| *T. monococcum* transplanted to: | | | | |
| *T. monococcum* | 0 | 0 | 0 | 0 |
| *T. durum* (Stewart) | 0 | 0 | 0 | 0 |
| *T. vulgare* (Chinese Spring) | 0 | 0 | 0 | 0 |
| *S. cereale* (Prolific) | 0 | 0 | 0 | 0 |
| *H. vulgare* (O.A.C. 21) | 0 | 0 | 0 | 0 |
| *T. durum-A. elongatum* amphiploid | 0 | 0 | 0 | 0 |
| *A. sativa* (Vicar) | 0 | 0 | 0 | 0 |
| *T. durum* (Stewart) transplanted to: | | | | |
| *T. durum* (Stewart) | 0 | 21 | 41 | 62 |
| *T. monococcum* | 0 | 18 | 35 | 53 |
| *T. vulgare* (Chinese Spring) | 0 | 12 | 39 | 51 |
| *S. cereale* (Prolific) | 0 | 14 | 55 | 69 |
| *H. vulgare* (O.A.C. 21) | 0 | 7* | 38 | 45 |
| *T. durum-A. elongatum* amphiploid | 2 | 7* | 29 | 38* |
| *A. sativa* (Vicar) | 0 | 16 | 31 | 47 |
| *T. Timopheevi* transplanted to: | | | | |
| *T. Timopheevi* | 2 | 22 | 59 | 83 |
| *T. vulgare* (Chinese Spring) | 1 | 39* | 78 | 118* |
| *S. cereale* (Prolific) | 4 | 23 | 62 | 89 |
| *H. vulgare* (O.A.C. 21) | 1 | 24 | 68 | 93 |
| *T. durum-A. elongatum* amphiploid | 4 | 28 | 98 | 130* |
| *T. vulgare* (Kenya Farmer) transplanted to: | | | | |
| *T. vulgare* (Kenya Farmer) | 0 | 0 | 2 | 2 |
| *T. vulgare* (Chinese Spring) | 0 | 0 | 2 | 2 |
| *S. cereale* (Prolific) | 0 | 0 | 0 | 0 |
| *T. vulgare* (Prelude) transplanted to: | | | | |
| *T. vulgare* (Prelude) | 10 | 7 | 1 | 18 |
| *T. monococcum* | 9 | 5 | 2 | 16 |
| *T. durum* (Stewart) | 14 | 13 | 7 | 34 |
| *T. vulgare* (Chinese Spring) | 13 | 4 | 5 | 22 |
| *S. cereale* (Prolific) | 5 | 15 | 7 | 27 |
| *H. vulgare* (O.A.C. 21) | 5 | 10 | 3 | 18 |
| *T. durum-A. elongatum* amphiploid | 12 | 11 | 8 | 31* |

*Indicates significant difference ($P<.05$) between crosses involving the control and those involving a specific transplant.

(5) transplantation of wheat embryos to endosperms of *T. vulgare* (Chinese Spring) were made. Furthermore, selected wheat embryos were transplanted to endosperms of *T. durum* (Stewart), *T. monococcum* and such alien endosperm types as *Hordeum vulgare* (O.A.C. 21), *Avena sativa* (Vicar) and *T. durum-A. elongatum* amphiploids.

The different series of embryo transplantations made in 1957 are shown in Table 1. The diploid, tetraploid or hexaploid wheat variety, when it was transplanted to its own endosperm, was considered to be the control. In 1958 the arrangement of the test was changed (Table 2). *T. mono-*

*coccum* was used exclusively for transplantations. Not transplanted *T. monococcum* and *T. monococcum* embryo on *T. monococcum* endosperm plants were used as controls. *T. vulgare* (Prelude) and *T. durum* (Stewart) were known to cross with "Prolific" rye (Table 1). Both varieties were sown without being transplanted and were used for control crossings. Seed set in these crosses should give indication about environmental conditions at crossing time.

Seeds of "embryo" and "endosperm" varieties were selected for normally developed, medium-sized grains. All seeds which were selected for transplantations and controls were sterilized by shaking the seed for 2 minutes in a 60 per cent methyl alcohol followed by immersion in a 0.1 per cent mercuric chloride solution for 4 minutes. The treated seeds were rinsed several times and finally soaked in distilled water for 6 to 12 hours. A few minutes before transplantation they were placed on filter paper. The embryo was removed from the endosperm variety with a single cut of a sterilized scalpel or safety-razor blade. The embryo to be transplanted was separated from its endosperm by cutting around the scutellum, then lifted up and transferred to the new endosperm. The addition of a drop of distilled water allowed the embryo to slip easily into position and fastened it to the endosperm without the use of a special adhesive, provided that the endosperm was sufficiently softened by water.

The reconstructed seeds were allowed to dry on filter paper for at least 12 hours after which they could be handled like normal seeds. An examination of this seed 10 days after planting showed that the embryo still adhered to its new endosperm. The average percentage germination of the "transplanted" seed was 95 per cent.

The prepared seeds were then sown in the field in the spring. Several planting dates were used to ensure sufficient plants for crossing over a period of time. Crossing was performed by removing the anthers from the female parent, bagging the emasculated heads, and replacing the anthers 1 to 3 days later with anthers from the male parent. As far as possible, emasculations and pollinations were carried out under similar conditions for all plants. In order to test as many plants as possible only one head from each plant was used.

In 1957, 20 centrally placed florets in each of 10 heads were emasculated and pollinated for each combination. In 1958, 20 florets on each of 25 heads were used for each combination. The weather conditions at crossing time were favourably warm and dry in both years.

In 1957 and 1958 the heads from the cross *T. monococcum* x *S. cereale* (Prolific) were examined 18 to 25 days after pollination. The seeds from the combinations *T. durum* (Stewart) x *S. cereale* (Prolific) (in 1957 and 1958) and *T. Timopheevi* and *T. vulgare* (Kenya Farmer) x *S. cereale* (Prolific) (in 1957) were harvested and classified 19 to 26 days after pollination. Embryos were placed on a culture medium (14). In 1957 and 1958 the seeds from the cross *T. vulgare* (Prelude) x *S. cereale* (Prolific) were harvested in the dry or mature stage 6 to 8 weeks after pollination. They were examined and embryos from aborted seeds were placed on culture medium.

Anything was counted as "seed" and listed under "Apparent Seed Set" which 3 weeks or more after pollination showed a seed coat, an endosperm and/or an embryo. It was found that seeds did not always develop both an embryo and an endosperm. The classification chosen was based on the fact that a seed to be viable must contain an embryo. Seeds with embryos were listed under "Viable Seeds". Within this group seeds having fully developed embryo and endosperm were classified as "Normal" while seeds which had ceased growth before development was completed were classified as "Aborted". "Inviable Seeds" include those seeds which contained an endosperm only. Statistical tests were applied according to the Chi-square ($x^2$) method.

## RESULTS

The germination of the transplanted seeds was slower than that of not transplanted seeds. Nevertheless, all plants appeared normal in growth habit and morphology. Slight differences in maturity were observed between plants of the same variety grown on different endosperm. A similar condition was noted by Mathon and Stroun (10) in their material.

The results of the crosses are given in Tables 1 and 2. The cross, *T. monococcum* x *S. cereale*, failed to set seed in either year (Tables 1 and 2). This was the case regardless of whether the embryos of *T. monococcum* were grown on their endosperm or were transplanted to endosperms from other species.

In this experiment relatively high seed yields were obtained from crosses of tetraploid wheat and rye which have previously been reported to set seed only occasionally (Tables 1 and 2). In 1957 the control group from the cross *T. durum* x *S. cereale* yielded an average of 62 apparent seeds, of which 21 were viable. The apparent seed set ranged from 38 to

TABLE 2. — SEED SET IN CROSSES OF TRANSPLANTED DIPLOID AND NORMAL TETRAPLOID AND HEXAPLOID WHEATS WITH *S. CEREALE* (PROLIFIC) IN 1958

| Female Parent | Apparent Seed Set | | | |
|---|---|---|---|---|
| | Viable Seeds | | Inviable seeds | Seeds |
| | Normal | Aborted | | |
| *T. monococcum* untransplanted | 0 | 0 | 0 | 0 |
| *T. monococcum* transplanted to: | | | | |
| *T. monococcum* | 0 | 0 | 0 | 0 |
| *T. durum* (Stewart) | 0 | 0 | 0 | 0 |
| *T. vulgare* (Prelude) | 0 | 0 | 0 | 0 |
| *S. cereale* (Prolific) | 0 | 0 | 0 | 0 |
| *H. vulgare* (O.A.C. 21) | 0 | 0 | 0 | 0 |
| *T. durum* (Stewart) untransplanted | 4 | 36 | 320 | 356 |
| *T. vulgare* (Prelude) untransplanted | 102 | 2 | 16 | 120 |

TABLE 3. — INFLUENCE OF EMBRYO SOURCE ON EFFECT OF EMBRYO TRANSPLANTATION IN WHEAT-RYE CROSSES

| Female Parent | Viable Seeds | | Apparent Seed Set | |
|---|---|---|---|---|
| | Trans-plants | Control | Trans-plants | Control |
| On *S. cereale* (Prolific) endosperm | | | | |
| *T. durum* (Stewart) | 14 | 21 | 69 | 62 |
| *T. Timopheevi* | 27 | 24 | 89 | 83 |
| *T. vulgare* (Prelude) | 20 | 17 | 27 | 18 |
| On *T. vulgare* (Chinese Spring) endosperm | | | | |
| *T. durum* (Stewart) | 12 | 21 | 51 | 62 |
| *T. Timopheevi* | 40 | 24* | 118 | 83* |
| *T. vulgare* (Prelude) | 17 | 17 | 22 | 18 |
| On *H. vulgare* (O.A.C. 21) endosperm | | | | |
| *T. durum* (Stewart) | 7 | 21* | 45 | 62 |
| *T. Timopheevi* | 25 | 24 | 93 | 83 |
| *T. vulgare* (Prelude) | 15 | 17 | 18 | 18 |
| On *T. durum-A. elongatum* amphiploid endosperm | | | | |
| *T. durum* (Stewart) | 9 | 21* | 38 | 52* |
| *T. Timopheevi* | 32 | 24 | 130 | 83* |
| *T. vulgare* (Prelude) | 23 | 17 | 31 | 18* |
| On *T. monococcum* endosperm | | | | |
| *T. durum* (Stewart) | 18 | 21 | 53 | 62 |
| *T. vulgare* (Prelude) | 14 | 17 | 16 | 18 |

*Indicates significant difference ($P < .05$) between comparable crosses of transplanted and normal wheat with rye.

69, but included only 7 to 18 viable seeds when the cross involved plants grown from *T. durum* embryos transplanted to other endosperms. The number of viable seeds in this cross appeared to be significantly lower than when *H. vulgare* (O.A.C. 21) endosperms had been used ($P < 0.5$). The number of apparent and viable seeds was significantly lower when plants were grown on *T. durum-A. elongatum* amphiploid endosperms ($P < .05$). Crosses of plants derived from *T. Timopheevi* embryos and endosperms (control group) with *S. cereale* produced an apparent seed set of 83, of which 24 were viable. For this cross apparent and viable seed sets were usually higher when embryos of the wheat parent had been transplanted to other endosperms. The number of apparent seeds formed in crosses involving such plants ranged from 89 to 130, that of viable seeds from 25 to 40. Both the apparent and the viable seed set seemed to be significantly increased when *T. Timopheevi* embryos were transplanted to endosperms of *T. vulgare* (Chinese Spring) ($P < .05$). The apparent seed set only was significantly increased when *T. Timopheevi* embryos were grown on *T. durum-A. elongatum* amphiploid endosperms ($P < .05$).

Crosses of hexaploid *T. vulgare* (Prelude) with rye were usually successful regardless of the derivation of the wheat parent. The apparent seed set from crosses of *T. vulgare* transplants x *S. cereale* ranged from

16 to 34. It was significantly greater than that of the control only when transplants to *T. durum-A. elongatum* amphiploid endosperms were used. The viable seed set ranged from 14 to 27. In no instance was it significantly different from the number of 17 viable seeds obtained from *T. vulgare* control x *S. cereale*. The cross of hexaploid *T. vulgare* (Kenya Farmer) with *S. cereale* was not successful. An apparent seed set of 3 was obtained when the wheat parent had been grown on its own or "Chinese Spring" endosperm, but in both cases these seeds were inviable.

A measure of the influence of the endosperm source on the seed set of crosses of wheat transplants with rye may be obtained from Table 3. The differences in seed set between transplants x *S. cereale* and control x *S. cereale* are usually small and show no consistent association with endosperm types. The significant increase in both apparent and viable seed set associated with transplants of *T. Timopheevi* to "Chinese Spring" endosperm only is limited to the cross involving *T. Timopheevi* only. Similarly a significant decrease in seed set is associated specifically with transplants of *T. durum* to *T. durum-A. elongatum* amphiploid endosperms.

## DISCUSSION

It is apparent that embryo transplantation has not markedly affected the number of seeds in this material (Tables 1, 2 and 3). For purposes of comparison crosses of rye with the diploid, tetraploid and hexaploid wheat, transplanted to its own endosperm, may be regarded as controls. The effect of embryo transplantation is characteristic for the cross and does not reflect association with specific endosperm sources (Tables 1 and 3). In crosses with rye transplants of *T. durum* (Stewart) produced fewer seeds than the *T. durum* control. Significantly lower seed yields were obtained when the endosperm source was *H. vulgare* (O.A.C. 21) but not all plants started on this endosperm showed a decreased seed set in crosses. Similarly, although a significantly higher number of viable seeds was obtained only when the *T. Timopheevi* embryo had been transferred to the endosperm of "Chinese Spring" wheat, all crosses involving *T. Timopheevi* transplants equalled or exceeded the control in the seed set.

The proposed transplantation method (12, 5) should influence incompatibility factors (2, 13) which are responsible for unsuccessful crosses. It has been noted from fruit trees and other crops (1) that stocks can have a temporary influence on vigour of grafts. In the case of embryo transplantation such "grafting effect" could be expected when embryos are transplanted to endosperms which are superior to their own endosperm in quality or quantity.

The effect of embryo transplantation appears of some significance when considered in terms of "apparent seed set". However, classification of this group shows that the method has had virtually no effect on the number of fully developed seeds and only a little on the total number of viable seeds. The lack of correlation between the apparent seed set and the number of viable seeds points out the fallacy of reporting only apparent seed set. The failure of embryo transplantation to increase the number of fully developed seeds means that embryo transplantation by

itself has little value. The use of this method in conjunction with embryo culture techniques may be a means of increasing the frequency of obtaining viable seeds from crosses of partially compatible species.

From the results in our experiments it is obvious that the wheat species used are of two different types:

(a) In the crosses involving *T. monococcum* and *T. vulgare* (Kenya Farmer) x *S. cereale* (Prolific), no viable seed was obtained in either the transplanted or the untransplanted groups.

(b) In the crosses involving *T. durum* (Stewart), *T. Timopheevi* and *T. vulgare* (Prelude), viable seeds were obtained either in the transplanted or in the not transplanted groups (Tables 1 and 2).

Leighty and Sando (7) and Taylor and Quisenberry (16) suggested that the success of wheat-rye crosses depends upon crossability genes in the wheat parent. The action of the crossability genes which are known to be associated with "Chinese Spring" wheat (8, 9, 16) must be such that it makes fertilization and the development of a viable seed possible. Therefore, it may be concluded that crossability genes are lacking in the wheat species of (a) and that those of (b) must contain crossability genes similar to those found in "Chinese Spring" wheat.

The most significant observation in this study is that *T. monococcum* and *T. vulgare* (Kenya Farmer) do not set seed in crosses with rye even after embryo transplantation. This treatment has not facilitated crossing of these species which had previously been classified as incompatible. The addition of endosperm from "Chinese Spring" and "Stewart" which are known to carry crossability genes was ineffective in overcoming the incompatibility barriers in these species. This implies that the main effect of these genes is not resident in the endosperm and cannot be added to another species by embryo transplantation.

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# THE PRECISION OF FIELD EXPERIMENTS WITH VEGETABLE CROPS AS INFLUENCED BY PLOT AND BLOCK SIZE AND SHAPE.

## II. CANNING PEAS[1]

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### ABSTRACT

In 1957, vine and shelled pea weights of canning peas from an irrigated uniformity trial were recorded to determine the effect on yield variability of varying plot and block sizes and shapes. The most uniform reduction in variation occurred in block shapes of one plot long and six plots wide with each increase in plot length. These results agree with those of other workers, that long, narrow blocks are more efficient than square blocks. The optimum plot size was found to be 5 feet long and 10 feet wide. Considerably more shelled peas were required for processing than could be obtained from the optimum size of plot for yield.

### INTRODUCTION

In a previous study (2) it was noted that field research with vegetable crops is hampered by the scarcity of adequate information regarding optimum plot sizes and shapes. This has resulted in wide range of plot sizes and shapes being chosen arbitrarily and, in many instances, without adequate information. Such important factors as soil variability and cost have not always been considered seriously when vegetable crop experiments have been designed. Thus many experiments in this field of research have been accompanied by reduced plot efficiency, and in some cases complete failure could be attributed to faulty experimental design.

A comprehensive review of the literature, as well as the details of the statistical methods, are presented in an earlier paper (2). No work of this type has been reported on canning peas.

Smith (5) showed that, by plotting the coefficient of variation against plot size, the point on the curve selected as optimum for plot size and shape was dependent on the unit of measure for the ordinate and the abscissa of the graph. To offset this difficulty he demonstrated that the regression of variability on plot size become linear when plotted on logarithmic paper and could be more easily interpreted. This means that the relative reduction in variability for a relative increase in plot size is similar throughout the observed range, i.e., doubling plot size results, on the average, in a proportional reduction in variability. The relationship was described by the formula $Vx = V_1/n^b$ where Vx is the variance of yield per unit area of plot composed of n units of area, $V_1$ is the variance of yield of the basic plot size, and b is the exponent that measures soil heterogeneity and correlation between adjacent plots.

Estimates of cost were obtained by Robinson *et al.* (4) indicating the percentage of the total cost that was proportional to the number of plots used and the percentage that was proportional to the total area used per treatment. Assuming a desired degree of accuracy (Sx = 3.5 per cent), the

[1]Contribution from the Horticulture Section. Part of a thesis submitted to the Graduate School, Oregon State College, Corvallis, Oregon, June 1958, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

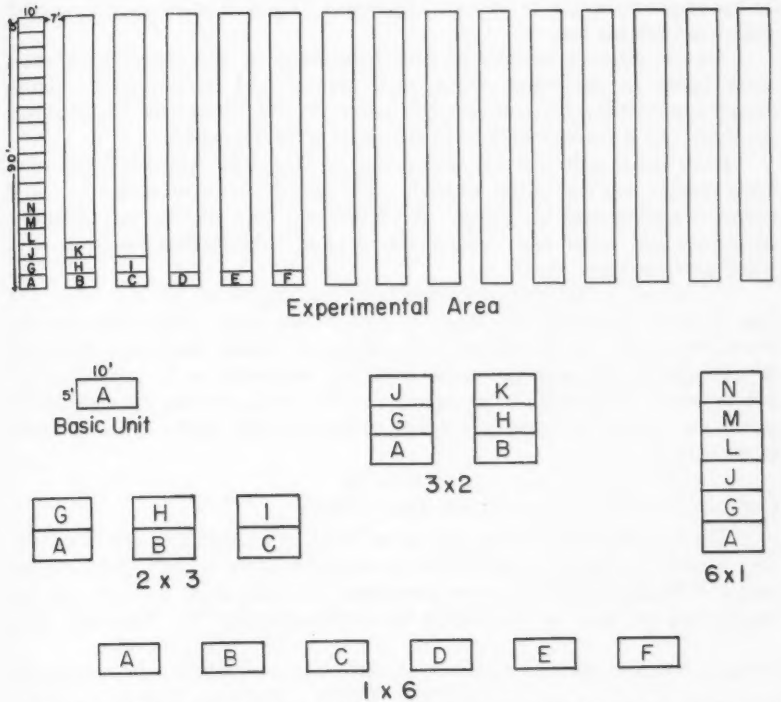


FIGURE 1. Diagrammatic sketch showing row arrangement within the experimental area, the basic unit, and the arrangement of the four block shapes of the canning pea uniformity trial.

relative cost for various plot dimensions was calculated and the optimum plot size indicated.

This study utilizes data from a uniformity trial with canning peas for the estimation of optimum plot sizes and shape. The data were obtained from two identical uniformity experiments with canning peas.

## MATERIALS AND METHODS

Two experimental areas were seeded at the rate of 240 pounds of peas per acre. The area selected had been planted to tomatoes the previous year and for the 2 years prior to that had been treated as follows: sown to grain the first year, green manure and 40-60 pounds per acre of ammonium-phosphate fertilizer the year prior to tomatoes, together with partial fallow. Each experimental area consisted of 15 rows 10 feet wide and 90 feet long, with the basic unit 5 feet long and 10 feet wide. Plots were composed of various combinations of basic units in a row (Figure 1). The rows were separated by a 7-foot bare strip to facilitate harvesting of the plots. The two areas were seeded on different days to avoid maturation on the same day. Germination was uniform. The row ends were trimmed

to the required length of 90 feet. Standard irrigation practices for canning peas were carried out.

Several random samples of peas from each of the experimental areas were taken to determine when each area should be harvested. These samples consisted of about 40 pods taken to determine the tenderometer reading. At a reading of 90-110 the plots were harvested.

Each basic unit was harvested with a Model G Allis-Chalmers row-crop tractor with a 5-foot mower. The weeds were extracted by hand before weighing and bunching. A record was kept of the time necessary to harvest and weigh each 5-foot unit of vines. Altogether 540 basic units were harvested and vined.

An experimental plot-size viner built and improved for this study (3) was used for handling the vines of each 5-foot unit. The time required for vining each 5-foot unit and the weight of shelled peas were recorded. A uniform loss of peas, per basic unit, was estimated to be approximately 3-4 per cent, due largely to immaturity of the peas, cracking by the paddles of the viner, and bouncing of the shelled peas as they struck the drop-apron of the viner.

## RESULTS

### *Comparison of Plot Lengths and Block Shapes*

The handling of canning peas in the field, as indicated under "Materials and Methods", made it impossible to combine plots in any direction but length. Blocks, however, were composed of basic units in each row for length and of rows of basic units for width (Figure 1). The data from

TABLE 1. — COEFFICIENTS OF VARIATION OF PEA YIELDS FOR VARYING PLOT LENGTHS AND BLOCK SHAPES

| Plot length 5′ units | Block shape | | Number of blocks | Coefficient of variation | |
|---|---|---|---|---|---|
| | Length plots | Width rows | | Pea vines (%) | Shelled peas (%) |
| 1 | 6 | 1 | 45 | 15.0 | 21.1 |
| 2 | 6 | 1 | 15 | 13.3 | 18.1 |
| 3 | 6 | 1 | 15 | 13.7 | 20.6 |
| 1 | 3 | 2 | 42 | 15.5 | 16.7 |
| 2 | 3 | 2 | 21 | 14.2 | 18.1 |
| 3 | 3 | 2 | 14 | 14.1 | 18.9 |
| 6 | 3 | 2 | 7 | 12.5 | 16.7 |
| 1 | 2 | 3 | 45 | 13.2 | 20.2 |
| 2 | 2 | 3 | 20 | 12.7 | 16.7 |
| 3 | 2 | 3 | 15 | 13.9 | 18.9 |
| 6 | 2 | 3 | 5 | 12.8 | 15.6 |
| 9 | 2 | 3 | 5 | 10.5 | 14.4 |
| 1 | 1 | 6 | 36 | 14.9 | 20.3 |
| 2 | 1 | 6 | 18 | 13.0 | 17.3 |
| 3 | 1 | 6 | 12 | 12.3 | 16.4 |
| 6 | 1 | 6 | 6 | 10.2 | 13.1 |
| 9 | 1 | 6 | 4 | 9.6 | 11.3 |

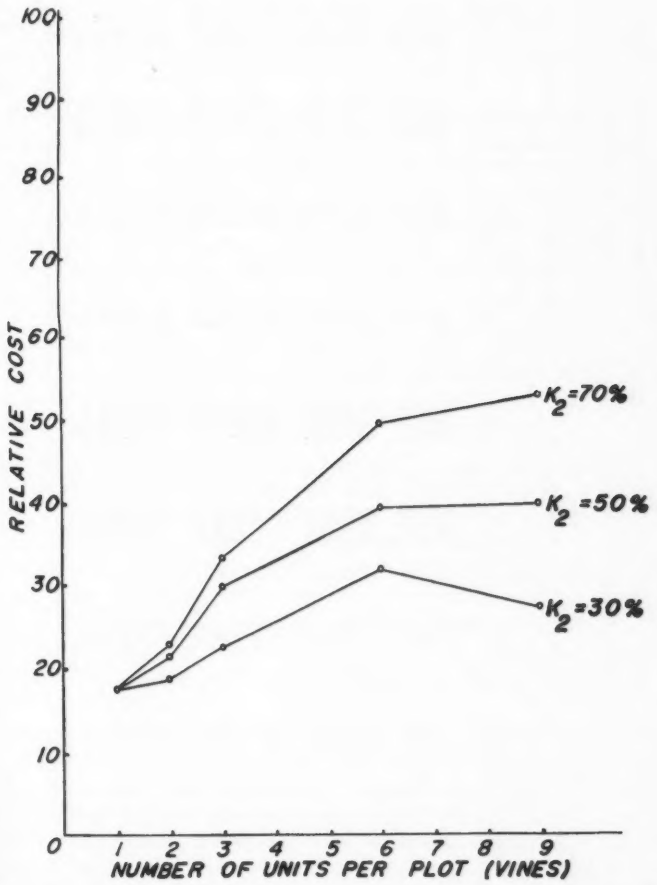


FIGURE 2. The relative cost of obtaining a desired degree of accuracy ($S\bar{x}$ = 3.5 per cent) using different plot lengths and block shapes with pea vines, assuming various percentages of the total cost to be proportional to the area used per treatment ($K_2$).

the two experimental areas were combined into one set, since they were side by side with no significant statistical differences noted between them.

Five different plot lengths were compared in four different block shapes. Wherever possible the entire uniformity data were utilized in the analysis. The coefficients of variation for vines and shelled peas were computed for evaluating various plot dimensions.

The coefficients of variation for vine and shelled pea data on a per-plot basis for 17 different plot and block combinations are given in Table 1.

It was impossible to utilize all of the experimental area for two of the four block shapes, namely, 3 plots long x 2 plots wide, and 1 plot long x 6 plots wide. The differences in variation with shelled peas were not as

TABLE 2. — EFFECT OF LENGTH OF PLOTS AND SHAPE OF BLOCKS IN EFFICIENCY OF CANNING PEA EXPERIMENTS

| Plot length 5′ units | Block shape | | Required replication[3] | | Area required per treatment | | | | Relative efficiency | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | No. of units | | Square feet | | Land use[4] | | Cost[5] | |
| | Length plots | Width rows | P.V.[1] | S.P.[2] | P.V. | S.P. | P.V. | S.P. | P.V. | S.P. | P.V. | S.P. |
| 1 | 6 | 1 | 18 | 36 | 18 | 36 | 900 | 1800 | 100 | 100 | 18.0 | 36.0 |
| 2 | 6 | 1 | 14 | 27 | 28 | 54 | 1400 | 2700 | 64 | 67 | 18.2 | 35.1 |
| 3 | 6 | 1 | 15 | 35 | 45 | 105 | 2250 | 5250 | 40 | 34 | 24.0 | 56.0 |
| 1 | 3 | 2 | 20 | 23 | 20 | 23 | 1000 | 1150 | 90 | 157 | 20.0 | 23.0 |
| 2 | 3 | 2 | 16 | 27 | 32 | 54 | 1600 | 2700 | 56 | 67 | 20.8 | 35.1 |
| 3 | 3 | 2 | 16 | 29 | 48 | 87 | 2400 | 4350 | 38 | 41 | 25.6 | 46.5 |
| 6 | 3 | 2 | 13 | 23 | 78 | 136 | 3900 | 6900 | 23 | 26 | 32.5 | 57.5 |
| 1 | 2 | 3 | 14 | 33 | 14 | 33 | 700 | 1650 | 129 | 109 | 14.0 | 33.0 |
| 2 | 2 | 3 | 13 | 23 | 25 | 46 | 1300 | 2300 | 69 | 78 | 16.9 | 29.9 |
| 3 | 2 | 3 | 15 | 30 | 45 | 90 | 2250 | 4500 | 40 | 40 | 24.0 | 48.0 |
| 6 | 2 | 3 | 13 | 20 | 78 | 120 | 3900 | 6000 | 23 | 39 | 32.5 | 50.0 |
| 9 | 2 | 3 | 9 | 16 | 91 | 144 | 4550 | 7200 | 22 | 106 | 30.6 | 54.0 |
| 1 | 1 | 6 | 18 | 34 | 18 | 34 | 900 | 1700 | 100 | 75 | 18.0 | 34.0 |
| 2 | 1 | 6 | 14 | 24 | 28 | 48 | 1400 | 2400 | 64 | 55 | 18.2 | 31.2 |
| 3 | 1 | 6 | 12 | 22 | 36 | 66 | 1800 | 3300 | 50 | 43 | 19.2 | 35.2 |
| 6 | 1 | 6 | 8 | 14 | 48 | 84 | 2400 | 4200 | 38 | 25 | 20.0 | 35.0 |
| 9 | 1 | 6 | 7 | 10 | 63 | 90 | 3150 | 4500 | 29 | 40 | 23.8 | 34.0 |

[1]Pea vine data
[2]Shelled pea data
[3]Replication required to reduce (S.E.) of a treatment mean to 3.5 per cent
[4]Ratio of area used by 5-foot single-row unit to the area used with larger plots x 100
[5]Assuming that of the total cost 70 per cent is proportional to the number of replications and 30 per cent to plot size

great as with vines. Nevertheless, as plot size increased, the trend was generally the same for both vines and shelled peas. The inconsistencies that did occur in variation were for the most part the same for both sets of data.

Increasing plot length from one to two units reduced variation in all block shapes except in the shelled pea data of block shapes of 3 plots long and 2 rows wide. Further increase in plot length in the block shapes of 1 plot long and 6 rows wide, and 2 plots long and 3 rows wide, showed little reduction in variation until the plots were six units long. However, in block shapes of 2 plots long and 3 rows wide, and particularly in block shapes of 1 plot long and 6 rows wide, there was a general reduction with increased plot length. Block shapes of 1 plot long and 6 rows wide, the widest blocks used, showed the most consistent and uniform reduction in variation with increasing plot length. However, lengthening the plots failed to cause the same reduction in variation as changing the block shapes. This serves to emphasize the need for careful consideration of plot layout within any block shape if variation is to be minimized.

Assuming a desired standard error of 3.5 per cent, and using the coefficients of variation for vines and shelled peas in Table 1, the number of replications required to attain this degree of accuracy is given in Table 2. In general the required number of replications decreased with increasing plot size. Land requirements per treatment increased rapidly as plot length increased; none of the inconsistencies noted under "Required replications" showed up. Under both "Replications" and "Area", shelled pea requirements were considerably larger than vines. This may have been due to the much smaller plot weight of the shelled peas than of the vines, which magnified technique errors.

Columns 10 and 11 of Table 2 give the relative efficiency of land use for vines and shelled peas. The basic plot length of block shapes of 6 plots long and 1 row wide was taken as the standard for comparison. Vine and shelled pea data do not agree as to the most efficient block shape. For vines the basic length in block shapes of 2 plots long and 3 rows wide gave the most efficient land use whereas with shelled peas the basic plot in block shapes of 3 plots long and 2 rows wide was the most efficient. Block shapes of 3 plots long and 2 rows wide of the vines and block shapes of 1 plot long and 6 rows wide of the shelled pea data showed the basic plot length to be less efficient in land use than the designated basic plot size. Block effect in plot efficiency is thus emphasized by these data.

In a previous study (2) it was pointed out that land occupied by a treatment in any experiment is only part of several items of cost. To determine these costs for canning pea uniformity experiments, a record was kept of the time and number of people required to perform the work. Assuming 30 per cent of the total cost is proportional to area, then the relative cost of plots one basic unit in length in block shapes of 2 plots long and 3 rows wide for vines should be (14) (.70) + (1) (14) (.30) = 14.0 and for shelled peas (33) (.70) + (1) (33) (.30) = 33.0, using the cost equation where relative cost = $rk_1 + nrk_2$ (2). The last two columns of

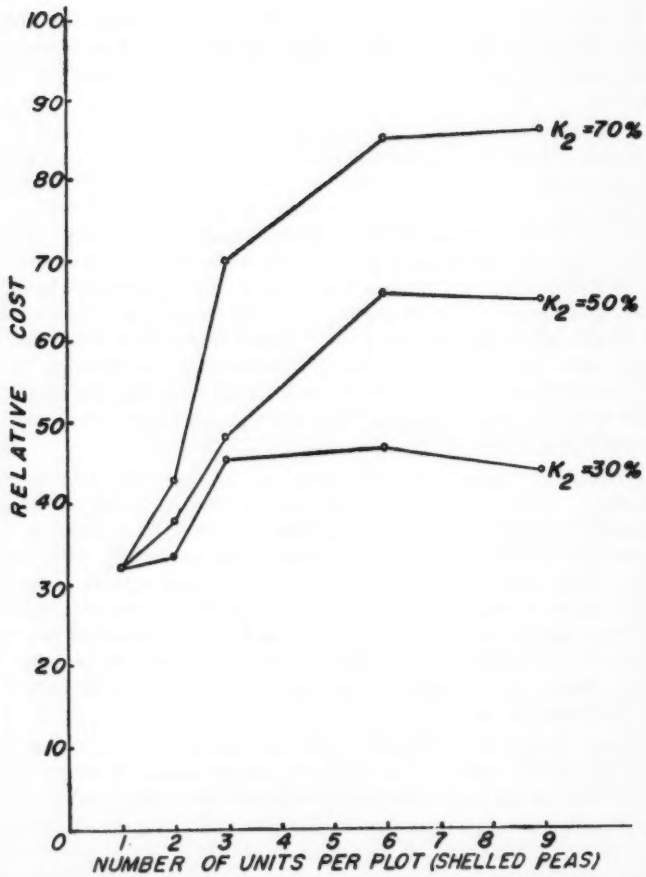


FIGURE 3. The relative cost of obtaining a desired degree of accuracy ($S\bar{x} = 3.5$ per cent) using different plot lengths and block shapes with shelled peas, assuming various percentages of the total cost to be proportional to the total areas used per treatment ($K_2$).

Table 2 give the relative cost of the various plot lengths and block shapes. The over-all pattern of these data indicates the most efficient size of plot is the smallest or basic unit.

Graphs depicting the relationship of relative cost and changing plot lengths for three different assumptions about the cost are given in Figures 2 and 3. The graphs show that, regardless of the cost assumptions, costs rise immediately from the basic unit. In the higher cost range, regardless of the assumptions of cost, there is a levelling off with increasing number of basic units per plot. Plots 9 units long are only slightly more efficient than plots of 6 units in length.

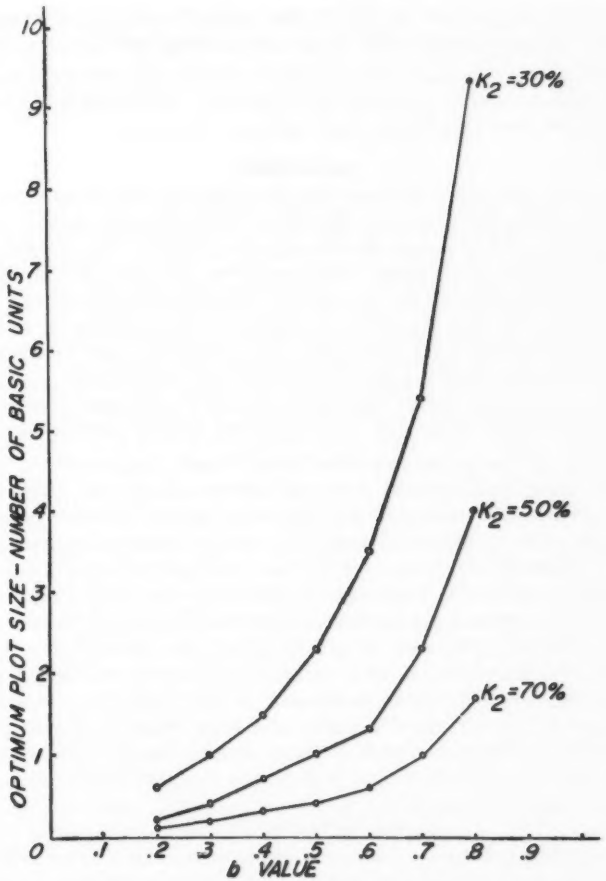


FIGURE 4. The effect on the optimum plot size of soil heterogeneity (b value) and the proportion of the cost of a treatment that is directly related to plot sizes ($K_s$).

*Comparison of Plot Length and Block Shapes Using Smith's Law*

Smith's empirical law (5) showed that, when the variance relationship was transformed to a logarithmic scale, the value of b could be obtained by a least squares estimate. For the vine data the regression coefficient b was .1913 and for shelled pea data .2296. The two b values showed little difference although obtained from two different sets of data.

The optimum size of plot for soils that differ in heterogeneity (expressed as b values) and for different assumptions about the percentage of the total cost that is proportional to plot size is shown in Figure 3. It can be readily seen that the data shown in Figures 4, 2, and 3 agree regarding

the most economical size of plot to use, namely, an optimum plot size of one unit. Thus the two methods of determining optimum plot size agree.

Using the actual $K_2n$ value of 38 per cent in the optimum size of plot equation (2), $n = bK_1/(1-b)K_2$, n = one plot. This result likewise agrees with the data shown in Figures 2, 3, and 4.

## DISCUSSION

In yield tests with canning peas lengthening the plots reduced variability regardless of block shape. The wider blocks showed a greater reduction in variability than did the longer blocks. The interplay of plot and block shapes arrangement was found by Moore and Darroch (1) to have an immediate effect on the over-all efficiency of experiments. Failure to compensate for this interplay of shapes in the planning of field experiments with canning peas may result in substantial loss in precision. It suggests also that vine weight and shelled pea weight are equally useful for recording plot weight. However, it would seem preferable to use the shelled pea weight since this represents the usable portion of the crop.

Quality studies are an important part of every experiment with canning peas. It is not sufficient to gauge treatment effects by yield alone. A previous study showed that it was necessary to bulk the shelled peas from nine basic units to obtain a minimum sample for quality studies. This meant that the optimum plot size of one basic unit for yield is inadequate for quality studies. To overcome this difficulty the yield from plot length that comes nearest to the actual requirement could be used for the treatment estimate. Table 2, columns 12 and 13, shows the most efficient lengths of plots to be longer than the basic unit. If, after using the larger plots, the variation was found to be high because of the necessity of splitting each plot sample for vining, the alternative would be to bulk the peas from each optimum-sized plot and use the bulked sample for quality measurements. The purpose of each experiment will dictate the method to use.

## ACKNOWLEDGEMENT

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# CHEMICAL COMPOSITIONS OF NATIVE FORAGE PLANTS IN BRITISH COLUMBIA IN RELATION TO GRAZING PRACTICES

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**ABSTRACT**

The percentages of crude protein and phosphorus decreased markedly and that of crude fibre increased in all classes of common forage in the southern interior of British Columbia from spring to fall on both grassland and forest ranges. No consistent trend was observed in percentage of total ash or calcium except that calcium increased markedly in weathered forbs both in the grasslands and in the forest. The grasses had a higher percentage of crude fibre than forbs or current shrub growth. The percentages of crude protein, crude fibre and phosphorus in a sedge meadow at a high elevation were all rather low during the grazing period; those of crude protein and phosphorus dropped sharply in mid-September. The chemical analyses indicated that the nutritive level of the range forage is generally satisfactory for livestock production although some winter supplementation, especially of phosphorus, may be necessary under certain conditions.

## INTRODUCTION

The great variety of topographic and climatic conditions occurring on the rangelands of British Columbia is associated with a corresponding variety of range types and plant species. Many species of grasses, some sedges, forbs, and shrubs, and even a few trees are foraged. Largely because of the topography, livestock are pastured at different elevations in different seasons on most of the ranges. This practice requires the periodic shifting of livestock from one range type to another and frequently involves marked changes in the kind and condition of forage available to livestock.

This is a report on chemical analyses of range forage plants from the interior of British Columbia obtained from 1935 to 1955. It represents the first major effort to determine the nutritive values of range forage in British Columbia.

The topography, climate, and vegetation of the principal range areas of the province have been described by Tisdale (12, 13) and Tisdale *et al.* (14).

In the Kamloops district the grazing season starts about mid-April, when the lower ranges are ready for use. The animals move into the higher grassland zones as the forage develops. The forest range is ready for use by mid-June and supplies good forage until the middle or end of September. The stock are then moved back to the winter ranges and meadows until exhaustion of the forage or heavy snows make feeding necessary.

In districts with major valleys, most livestock are wintered on river bottoms and benches, the climate being favourable and feed readily available. This situation prevails over most of the southern part of the ranching area. Further north, in the Cariboo and Chilcotin districts, favourable wintering spots are less common and many livestock are wintered on native meadows.

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Though availability of nutrients depends on their digestibility as well as their amount, chemical analyses have proved useful in indicating possible deficiencies of certain constituents. Among the minerals, phosphorus has most commonly been found deficient in amount, especially in weathered forage (2, 16). Deficiencies of other nutrients have been reported, although in many cases the degree of deficiency depends on the kind, class, and prior nutrition of livestock, as well as upon the content of the forage.

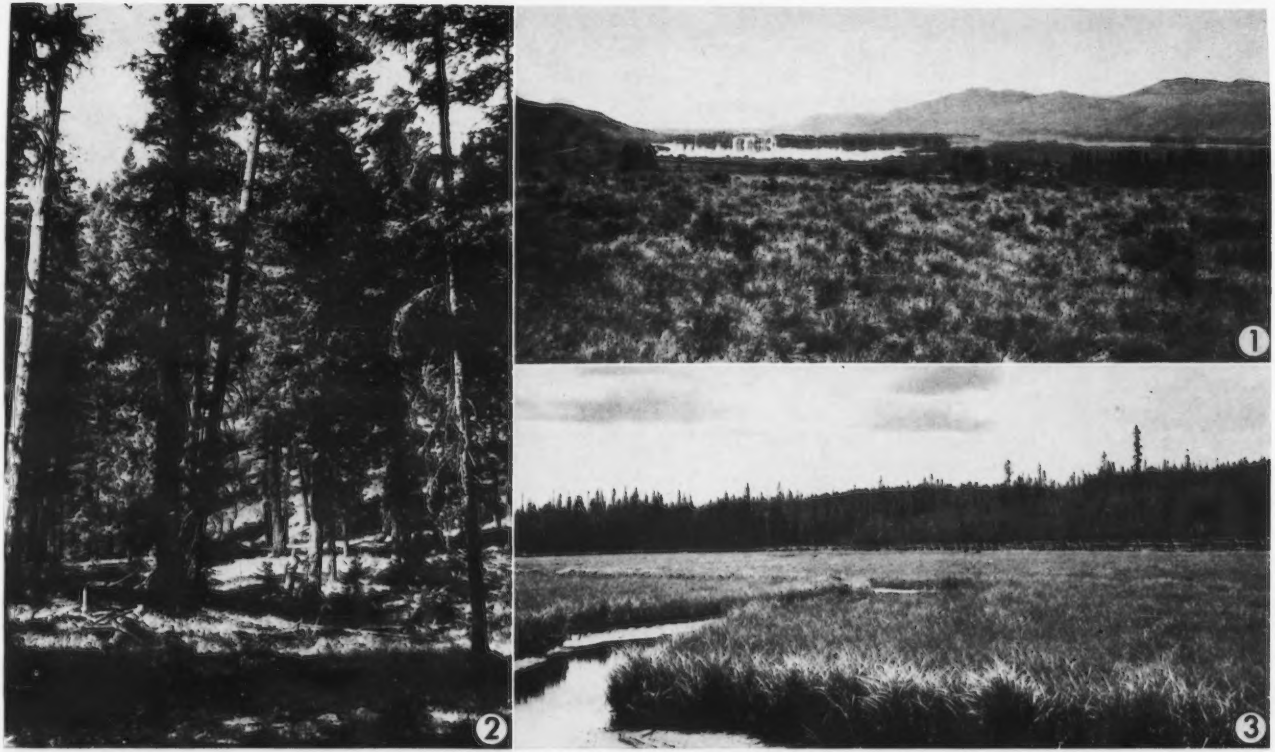
## VEGETATION OF THE STUDY AREA

The major zones of vegetation with increasing altitude in the range areas are the sagebrush-wheatgrass zone dominated by big sagebrush (*Artemisia tridentata* Nutt.) and beardless wheatgrass (*Agropyron inerme* (Scrib. & Smith) Rydb.) (Figure 1); the wheatgrass-bluegrass zone, dominated by beardless wheatgrass and Sandberg's bluegrass (*Poa secunda* Presl.); and the fescue-wheatgrass zone, dominated by rough fescue (*Festuca scabrella* Torr.) and beardless wheatgrass. Above the three grassland zones lie two forest zones, namely, Douglas-fir and spruce-alpine fir.

In the grasslands several other species, including Kentucky bluegrass (*Poa pratensis* L.), needle-and-thread (*Stipa comata* Trin. & Rupr.), Columbia needlegrass (*Stipa columbiana* Macoun), rough fescue, Junegrass (*Koeleria cristata* (L.) Pers.), and downy brome (*Bromus tectorum* L.) contribute important amounts of forage. Palatable forbs are scarce in the lower zones, but in the fescue-wheatgrass community, balsamroot (*Balsamorhiza sagittata* (Pursh) Nutt.), timber milk-vetch (*Astragalus decumbens* (Nutt.) Gray var. *serotinus* Gray), asters (*Aster* spp.), geranium (*Geranium viscosissimum* F. & M.), and vetch (*Vicia americana* Muhl.) furnish considerable forage.

The ponderosa pine zone is not well represented in most of the range region, and where present usually occurs as a relatively narrow strip between the grasslands and the Douglas-fir forest. Beardless wheatgrass, rough fescue, and Junegrass are the principal forage species.

Two major forest zones, Douglas-fir and spruce-alpine fir, make up the extensive summer forest grazing region. The Douglas-fir zone is the lower in elevation and is dominated by Douglas-fir (*Pseudotsuga menziesii* (Mirbel) Franco) (Figure 2), lodgepole pine (*Pinus contorta* Dougl. var. *latifolia* Engelm.), and/or aspen (*Populus tremuloides* Michx.). The undercover varies considerably with the nature and density of the tree stand, but it is generally dominated by pinegrass (*Calamagrostis rubescens* Buckl.) along with a variety of herbaceous plants including asters, lupine (*Lupinus glacialis* Smith), vetch, peavine (*Lathyrus ochroleucus* Hook.), milkvetch, and other forbs. The shrub cover is less well developed, the principal species being rose (*Rosa*), spirea (*Spiraea lucida* Dougl.), soopolallie (*Shepherdia canadensis* (L.) Nutt.), service berry (*Amelanchier alnifolia* Nutt.), and snowberry (*Symphoricarpos albus* (L.) Blake). Several species of willow (*Salix*) are common, especially in the earlier stages of succession following fire. Most of the herbaceous and shrubby species listed above are important as forage; pinegrass produces about 60 per cent of the herbage yield over large areas.



FIGURES 1—3. FIGURE 1. Grassland range at 1400-foot altitude near Kamloops, B.C. FIGURE 2. Douglas-fir forest range at 3300-foot altitude near Kamloops, B.C. FIGURE 3. Typical sedge meadow at 4000-foot altitude, Tranquille range near Kamloops, B.C.

The spruce-alpine fir zone is extensive, but over much of its area the undercover is relatively sparse and is composed mainly of dwarf shrubs of low palatability to livestock. Livestock grazing is largely confined to the numerous sedge meadows, and to parklike openings in the upper portions of the zone. The meadows are dominated by sedges (*Carex*) and rushes (*Juncus*) along with lesser amounts of certain grasses, especially marsh reedgrass (*Calamagrostis canadensis* (Michx.) Beauv.) (Figure 3). These meadows are often extensive and furnish large amounts of moderately palatable forage.

An additional range type, limited in area but extremely important in the annual nutritional pattern for livestock, is furnished by the lowland river bottoms. These occur mainly in the river valley bottoms and include both native and cultivated types. They are dominated mainly by sedges (*Carex*), but contain considerable variety of other plants, including horsetail (*Equisetum*).

## GENERAL METHODS

Samples of 17 grassland and 16 forest species were collected in various growth stages from different vegetation zones, principally in the Kamloops district, from 1935 to 1940 and from 1950 to 1955. From 3 to 12 samples, each 1- to 2-pounds green weight, were taken for each growth stage of each species, the number depending on the variation in results and on the relative importance of the species. A sample usually consisted of one species in a single growth stage but a few composite samples were taken. Herbaceous species were clipped at approximately the same heights as those grazed by cattle, but beyond this no attempt was made to segregate the parts of plants grazed most or least by livestock. Of shrubs, only the current year's growth was clipped. Each sample was air-dried, ground, and mixed, and a portion taken for chemical analysis. The analyses were made by officers of the Chemistry Division, Science Service, Canada Department of Agriculture, Ottawa. All samples were analysed for crude protein, crude fibre, total ash, calcium, and phosphorus; those collected after 1950 were analysed for ether extract also. All results are reported on a dry-matter basis.

In the sagebrush-wheatgrass and wheatgrass-bluegrass zones, grasses provide over 90 per cent of the total volume of forage. The remainder is supplied by a variety of forbs and a few shrubs. Hence the forb and shrub groups were not studied in detail in these zones. In the fescue-wheatgrass community, forbs and shrubs produce an appreciable part of the forage; no one species, however, is outstanding in this respect except balsamroot. Because of the great variety of forbs within this zone, only a few representative species were selected for study. A number of species sampled in the forest range are also important in the fescue-wheatgrass community, namely, timber milk-vetch, creamy peavine, vetch, rose, snowberry, and serviceberry. Most of the samples of these six species were collected within the forest range. Results from a number of samples collected in the grasslands, however, indicated that their compositions were within the limits for those that were collected on forest range.

TABLE 1—CHEMICAL COMPOSITIONS (PERCENTAGES, OVEN-DRY BASIS) OF NINE PRINCIPAL GRASSES OF THE GRASSLAND RANGES AT VARIOUS STAGES OF GROWTH

| Species | Crude protein | | | | Crude fibre | | | | Total ash | | | | Average collection date | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered |
| *Agropyron inerme* | 15.01 | 8.68 | 6.54 | 3.08 | 27.69 | 31.87 | 32.26 | 36.84 | 11.61 | 8.64 | 7.19 | 8.85 | May 9 | June 15 | June 29 | Nov. 6 |
| *Bromus tectorum* | 19.69 | 7.75 | 5.70 | 2.80 | 20.07 | 25.00 | 28.56 | 39.48 | 10.79 | 6.47 | 5.58 | 4.42 | Apr. 27 | May 28 | Aug. 7 | Oct. 29 |
| *Festuca scabrella* | 10.54 | 7.27 | 4.83 | 2.05 | 32.11 | 33.88 | 37.67 | 42.47 | 13.31 | 15.16 | 9.86 | 10.16 | May 28 | July 25 | Aug. 8 | Nov. 16 |
| *Koeleria cristata* | 12.83 | 8.37 | 5.60 | 1.89 | 28.51 | 33.45 | 34.79 | 41.56 | 10.82 | 10.26 | 9.75 | 8.45 | May 14 | June 19 | July 21 | Oct. 24 |
| *Poa pratensis* | 17.20 | 8.34 | 4.70 | 3.13 | 20.79 | 31.13 | 33.82 | 39.25 | 9.78 | 8.73 | 8.53 | 7.42 | May 25 | June 17 | July 25 | Nov. 10 |
| *Poa secunda* | 21.33 | 10.53 | 5.59 | 3.38 | 20.16 | 29.56 | 33.72 | 42.30 | 12.05 | 7.32 | 5.45 | 3.68 | Apr. 24 | May 14 | July 27 | Nov. 5 |
| *Sporobolus cryptandrus* | 15.58 | 9.96 | 8.06 | 6.45 | 26.44 | 31.39 | 29.63 | 38.62 | 8.80 | 8.07 | 7.08 | 7.59 | June 4 | June 25 | Aug. 12 | Nov. 7 |
| *Stipa columbiana* | 18.09 | 10.08 | 6.57 | 2.97 | 23.68 | 33.19 | 35.01 | 49.62 | 7.65 | 5.53 | 6.87 | 4.56 | May 25 | June 16 | July 26 | Nov. 15 |
| *Stipa comata* | 13.09 | 7.83 | 6.52 | 3.56 | 25.77 | 32.99 | 31.81 | 34.28 | 9.89 | 6.62 | 9.56 | 7.63 | May 13 | June 7 | July 27 | Nov. 22 |
| Average | 15.92 | 8.75 | 6.01 | 3.25 | 25.02 | 31.38 | 33.03 | 40.40 | 10.52 | 8.53 | 7.76 | 6.97 | May 15 | June 16 | July 27 | Nov. 8 |

TABLE 1. (*Continued*)

| Species | Calcium | | | | Phosphorus | | | | Ca/P ratio | | | | Number of samples | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered |
| *Agropyron inerme* | .33 | .34 | .39 | .35 | .37 | .24 | .13 | .10 | .93 | 1.44 | 3.30 | 3.82 | 5 | 8 | 5 | 9 |
| *Bromus tectorum* | .65 | .33 | .28 | .21 | .53 | .24 | .14 | .04 | 1.28 | 1.44 | 2.25 | 4.60 | 3 | 5 | 4 | 5 |
| *Festuca scabrella* | .32 | .35 | .30 | .28 | .38 | .32 | .19 | .08 | .84 | 1.12 | 1.63 | 3.39 | 3 | 4 | 4 | 4 |
| *Koeleria cristata* | .41 | .33 | .39 | .28 | .33 | .29 | .20 | .08 | 1.22 | 1.13 | 1.95 | 3.33 | 3 | 5 | 4 | 3 |
| *Poa pratensis* | .22 | .18 | .23 | .23 | .44 | .27 | .18 | .11 | .54 | .69 | 1.34 | 2.40 | 3 | 4 | 5 | 4 |
| *Poa secunda* | .43 | .30 | .27 | .20 | .39 | .28 | .07 | .06 | 1.11 | 1.10 | 2.50 | 4.00 | 3 | 3 | 3 | 3 |
| *Sporobolus cryptandrus* | .34 | .36 | .33 | .30 | .30 | .28 | .27 | .12 | 1.13 | 1.30 | 1.46 | 2.64 | 3 | 4 | 5 | 4 |
| *Stipa columbiana* | .31 | .25 | .28 | .26 | .31 | .20 | .16 | .05 | 1.00 | 1.25 | 1.72 | 5.00 | 3 | 3 | 3 | 3 |
| *Stipa comata* | .31 | .22 | .37 | .40 | .26 | .17 | .14 | .07 | 1.23 | 1.25 | 3.05 | 6.97 | 5 | 5 | 7 | 6 |
| Average | .36 | .29 | .31 | .27 | .36 | .25 | .16 | .08 | 1.03 | 1.19 | 2.13 | 4.01 | | | | |

TABLE 2. — CHEMICAL COMPOSITIONS (PERCENTAGES, OVEN-DRY BASIS) OF EIGHT SECONDARY GRAZING SPECIES OF THE GRASSLAND RANGES AT VARIOUS STAGES OF GROWTH

| Species | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Crude protein | | | | Crude fibre | | | | Total ash | | | | Average collection date | | | |
| *Achillea lanulosa* | 18.71 | 9.30 | — | 5.01 | 16.74 | 32.87 | — | 37.26 | 12.81 | 8.35 | — | 7.02 | May 12 | July 3 | — | Sept. 20 |
| *Balsamorhiza sagittata* | 19.69 | 18.03 | 10.70 | 3.46 | 13.68 | 17.40 | 19.86 | 20.82 | 15.56 | 13.30 | 15.31 | 16.77 | May 17 | May 25 | June 30 | Sept. 12 |
| *Carex praegracilis* | — | 10.45 | 7.84 | 3.62 | — | 27.70 | 27.88 | 33.08 | — | 7.74 | 9.35 | 12.48 | — | June 15 | July 28 | Nov. 4 |
| *Chrysothamnus nauseosus* | 12.85 | 8.86 | 7.45 | — | 26.81 | 29.66 | 25.69 | — | 5.97 | 6.02 | 6.15 | — | May 14 | Aug. 23 | Sept. 30 | — |
| *Elymus cinereus* | 23.54 | 9.70 | 5.91 | 2.35 | 23.51 | 33.76 | 38.51 | 40.80 | 14.56 | 8.31 | 9.51 | 8.74 | May 10 | July 3 | July 25 | Nov. 4 |
| *Juncus balticus* | — | 10.66 | 6.40 | 3.48 | — | 30.48 | 33.87 | 44.16 | — | 6.29 | 6.11 | 3.08 | — | May 25 | Aug. 23 | Nov. 7 |
| *Oryzopsis hymenoides* | 17.24 | 9.68 | 7.44 | 4.46 | 24.74 | 30.85 | 32.36 | 42.12 | 8.00 | 6.54 | 7.26 | 7.18 | May 6 | June 11 | July 18 | Oct. 15 |
| *Stipa richardsonii* | 15.14 | 7.78 | 6.91 | 1.99 | 25.25 | 32.20 | 33.91 | 41.00 | 6.11 | 6.69 | 7.79 | 5.90 | May 22 | June 16 | July 24 | Oct. 25 |
| Average of nine herbs | 24.03 | 15.39 | 12.85 | 8.22 | 18.85 | 27.16 | 27.02 | 30.73 | 10.75 | 8.49 | 8.78 | 9.24 | May 19 | June 18 | Aug. 8 | Sept. 21 |
| Average of four shrubs | 18.23 | 11.46 | 9.68 | 4.81 | 16.08 | 19.38 | 18.40 | 30.31 | 6.56 | 6.64 | 6.94 | 4.65 | May 19 | July 16 | Aug. 21 | Oct. 16 |

TABLE 2. (*Continued*)

| Species | Calcium | | | | Phosphorus | | | | Ca/P ratio | | | | Number of samples | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered |
| *Achillea lanulosa* | .96 | .87 | — | 1.12 | .42 | .54 | — | .16 | 2.29 | 1.84 | — | 7.00 | 2 | 3 | — | 2 |
| *Balsamorhiza sagittata* | 1.58 | 1.30 | 2.05 | 3.02 | .64 | .50 | .67 | .49 | 2.48 | 2.66 | 3.28 | 7.52 | 3 | 4 | 6 | 3 |
| *Carex praegracilis* | — | .26 | .27 | .46 | — | .22 | .23 | .17 | — | 1.22 | 1.19 | 2.71 | — | 3 | 2 | 1 |
| *Chrysothamnus nauseosus* | .58 | .74 | .54 | — | .34 | .22 | .20 | — | 1.70 | 3.39 | 2.69 | — | 3 | 3 | 3 | — |
| *Elymus cinereus* | .44 | .17 | .22 | .21 | .37 | .26 | .17 | .09 | 1.11 | .65 | 1.48 | 2.14 | 2 | 2 | 1 | 2 |
| *Juncus balticus* | — | .29 | .32 | .44 | — | .21 | .12 | .04 | — | 1.39 | 2.67 | 10.70 | — | 3 | 1 | 2 |
| *Oryzopsis hymenoides* | .50 | .35 | .33 | .38 | .20 | .12 | .12 | .05 | 2.50 | 2.85 | 2.80 | 7.16 | 3 | 3 | 3 | 3 |
| *Stipa richardsonii* | .51 | .22 | .22 | .21 | .71 | .18 | .20 | .08 | .72 | 1.22 | 1.12 | 2.39 | 3 | 3 | 3 | 3 |
| Average of nine herbs | .94 | .94 | 1.37 | 1.78 | .45 | .40 | .34 | .24 | 2.12 | 2.62 | 4.73 | 8.48 | | | | |
| Average of four shrubs | .46 | .83 | .92 | .73 | .46 | .41 | .39 | .21 | 1.08 | 2.48 | 2.37 | 3.42 | | | | |

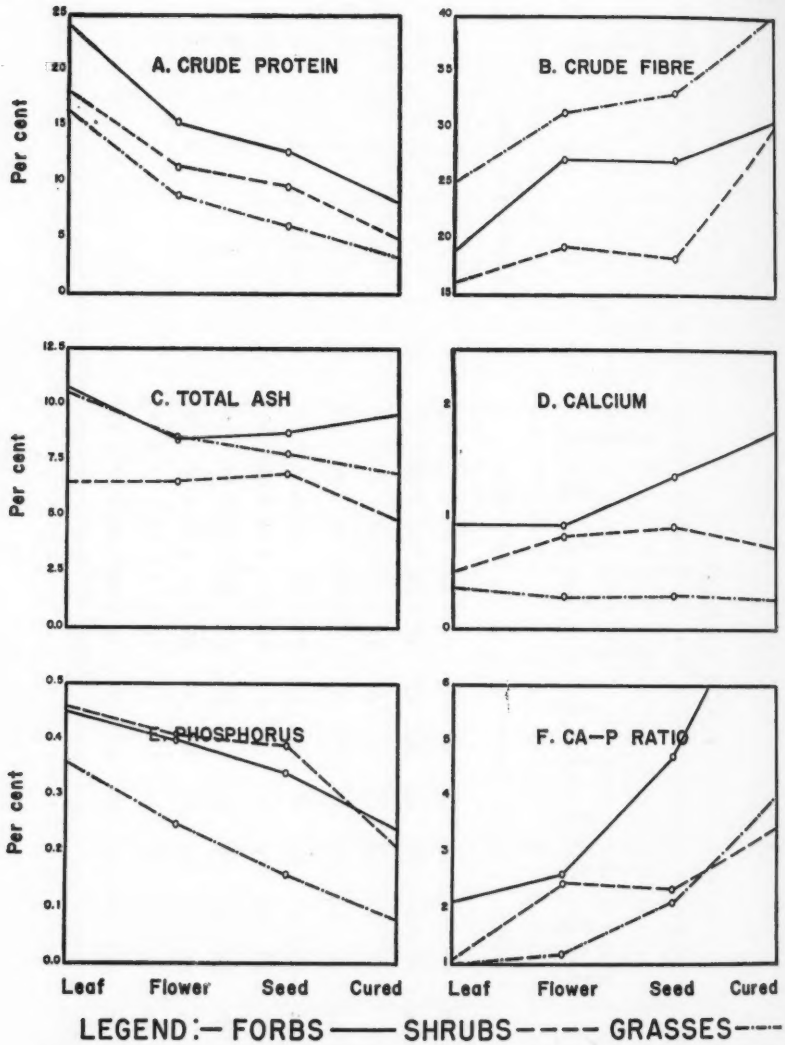



FIGURE 4. Chemical compositions of the three classes of forage of the grasslands at four growth stages (averages of 16 to 41 samples).

In the Douglas-fir forest range the most important single species is pinegrass, the only grass sampled. The forbs as a group are widely grazed, and the shrubs to a lesser extent.

Plots on a typical sedge meadow near Kamloops at an elevation of 4,800 feet were clipped at various intervals as part of a grazing capacity experiment.

To assess the chemical composition of well-cured native hay, samples were obtained from stacks at Kamloops, Tranquille, and the Lower Cariboo.

Of the important forage species, beardless wheatgrass, rough fescue, and pinegrass were selected for special study because of their important contribution to range livestock feed. A "seed shed" growth stage was added. Composite samples were taken from two of the forest range sites for comparison with the data from individual species on these sites.

A limited number of samples (one sample from each of three localities) of beardless wheatgrass and rough fescue were analysed for crude fat, nitrogen-free extract, and total carbohydrates.

Pinegrass is more readily eaten by cattle when growing in full sunlight than when growing in the shade of trees. To investigate whether this preference might be the result of different sugar contents of grass, samples were taken in two localities in early September from both sunny and shaded sites.

In assessing the nutritive value of the range forage certain common standards were used. The minimum protein in forage required for maintenance was taken as 6 per cent, for lactating animals 9 per cent, and for rapid growth 12 per cent (9). The maintenance requirements for calcium and phosphorus for mature cattle were taken as 0.25 and 0.12 per cent respectively (15). For growth, however, 0.20 per cent of phosphorus is required (9). The requirements for pregnant animals for both minerals would be considerably higher. Under most conditions the rates of calcium to phosphorus should be about 2 to 1, although wider ratios can be tolerated if the total phosphorus content is relatively high (15).

## GRASSLAND RANGE

The nutrient levels of the forage on grassland ranges should be adequate for all stock during the spring grazing season (Tables 1, 2). The forage for fall use, however, appears inadequate to provide maintenance levels of crude protein and phosphorus, especially since the forbs provide relatively less forage during this season because of withering and leaf drop (Figure 4). The somewhat high calcium-phosphorus ratio coupled with low total phosphorus may result in a phosphorus-deficient range during the late fall and winter.

## FOREST RANGE

Most of the forest range species are grazed during the late-leaf, flower and seed stages. By the time the plants are weathered in the fall the cattle are moving back on to the grassland ranges.

The rather high crude-fibre content of pinegrass combined with the low crude-protein levels (Tables 3, 4) suggests that this species leaves much to be desired as forage during most of the grazing period. In general, forage on the forest ranges probably does not meet animal growth requirements from about mid-August onwards but should meet maintenance requirements until cattle leave this range in the fall (Figure 5). For ranges consisting mostly of pinegrass, however, the values would be considerably lower. In the fall the phosphorus content is likely to drop below growth requirements on pinegrass range but should be sufficient for maintenance.

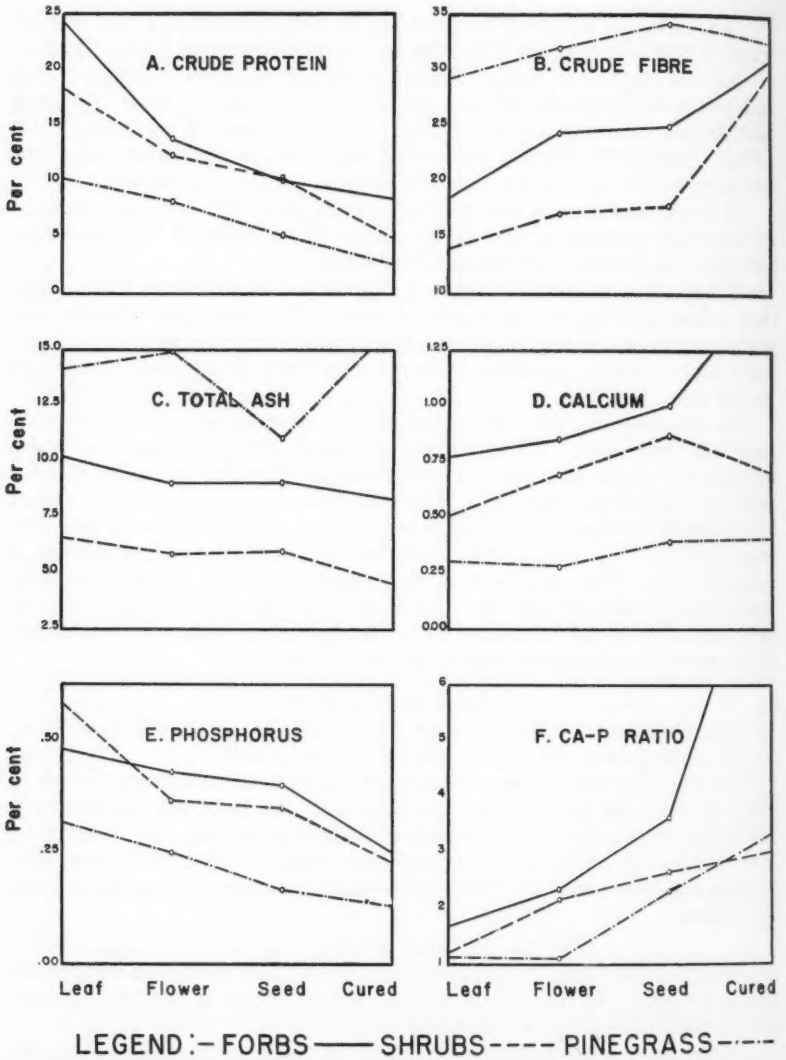



FIGURE 5. Chemical compositions of the three classes of forage of the forest range at four growth stages (averages of 8 to 28 samples).

The presence of forbs in composite samples that consisted largely of pinegrass was sufficient to raise the crude protein and calcium levels and the calcium-phosphorus ratio above those of pinegrass alone (Table 5).

Shade-grown pinegrass plants were slightly higher in sugar and starch (10.53 and 1.38 per cent respectively) than those grown in the sun (9.22

and 1.06 per cent respectively). There is no experimental evidence that the above differences explain the animals' preference.

### MOUNTAIN MEADOW RANGE

Much of the midsummer grazing in the spruce-alpine fir zone is to be found on wet meadows, which produce predominantly sedges (*Carex* spp.). These meadows are usually in drainage basins and are thus subject to spring and early-summer flooding. The meadows are usually grazed from June 15 to September 30.

The sedges on the meadows are close to physiological maturity by the end of July (Table 6). At this time the average crude protein scarcely met requirements for growing stock. The sharp drop in percentage protein during September is perhaps partly attributable to the severe frosts that occur at this time. The trend of phosphorus introduces the possibility that this nutrient may be a limiting factor in growth during the latter part of certain seasons, especially if prior nutrition of the animals has placed them in a borderline phosphorus deficiency state.

### WINTER FEED

The crude-protein values of all samples of native hay were sufficiently high to meet maintenance requirements (Table 7). The calcium content of hay consisting principally of sedges and horsetail was high. This condition, coupled with generally low phosphorus levels, resulted in unfavourable calcium-phosphorus ratios. Samples that consisted primarily of grasses and sedges should be good hay.

### GENERAL DISCUSSION

The changes in chemical composition of the various species during the season generally follow patterns similar to those found in other regions (2, 5, 7, 10, 16). Seasonal decreases in percentages of crude protein and phosphorus, and increases in crude fibre (or lignin and cellulose under the newer analytical methods) have been reported generally. Percentage of ether extract has been shown to decline in most cases, although the trend is usually less regular than for protein or phosphorus (2, 4, 5). No seasonal trends in calcium or total-ash contents were observed; similar observations have been reported by other workers (8, 10).

Appreciable differences in chemical compositions were observed between the three major classes of forage, namely, grasses, forbs, and browse. Grasses are lower in crude protein, ether extract, phosphorus, and calcium and higher in crude fibre than the forb or browse species studied in the same stage of seasonal development. Several other workers have reported on the superiority of browse over grasses in content of protein and phosphorus, especially during the fall and winter (1, 3).

As most grassland ranges in the interior of British Columbia are used only in spring and fall and the forest ranges in summer, the nutrient values of the forage must be considered on the basis of this seasonal use. This study indicates that the quality of the forage consumed was high at the beginning of the grazing period and with normal zonal grazing remained fairly high until about the end of July. The main reason for this is that the cattle are moved from lower to higher altitudes during the period and thus continue to graze on the earlier-growth stages of the main forage plants.

TABLE 3. — CHEMICAL COMPOSITIONS (PERCENTAGES, OVEN-DRY WEIGHT) OF NINE FOREST HERBS AT VARIOUS STAGES OF GROWTH

| Species | Crude protein | | | | Crude fibre | | | | Total ash | | | | Average collection date | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered |
| *Arnica cordifolia* | 19.19 | 10.80 | 6.19 | — | 12.15 | 17.72 | 13.49 | — | 12.18 | 12.72 | 15.05 | — | May 22 | June 18 | July 31 | — |
| *Aster conspicuus* | 23.00 | 11.36 | 8.14 | 4.53 | 14.68 | 22.43 | 22.64 | 25.44 | 13.87 | 11.56 | 10.39 | 10.70 | May 27 | July 7 | Aug. 20 | Oct. 2 |
| *Astragalus serotinus* | 20.38 | 12.60 | 11.61 | — | 21.92 | 29.25 | 30.06 | — | 7.76 | 5.48 | 5.36 | — | June 8 | June 19 | Aug. 16 | — |
| *Castilleja miniata* | 19.58 | 12.22 | 8.50 | 5.75 | 15.52 | 20.79 | 21.36 | 38.78 | 10.40 | 9.50 | 9.60 | 6.80 | May 22 | July 2 | July 27 | Oct. 16 |
| *Hieracium umbellatum* | — | 9.22 | 5.40 | — | — | 29.31 | 29.98 | — | — | 7.36 | 7.56 | — | — | July 22 | Aug. 3 | — |
| *Lathyrus ochroleucus* | 31.30 | 18.04 | 15.35 | 11.67 | 21.01 | 28.47 | 27.94 | 31.11 | 9.28 | 7.84 | 6.77 | 6.47 | May 22 | July 2 | Aug. 10 | Sept. 26 |
| *Lupinus glacialis* | 27.04 | 17.09 | 12.19 | 7.92 | 23.52 | 20.00 | 24.55 | 27.01 | 8.52 | 10.21 | 9.91 | 8.49 | June 8 | July 13 | Aug. 1 | Oct. 5 |
| *Vicia americana* | 30.06 | 19.01 | 13.76 | 12.76 | 20.91 | 27.82 | 30.23 | 33.74 | 8.35 | 7.50 | 7.70 | 6.70 | May 27 | July 2 | Aug. 13 | Sept. 28 |
| Average | 24.36 | 13.79 | 10.14 | 8.52 | 18.53 | 24.47 | 25.03 | 31.21 | 10.19 | 9.02 | 9.04 | 7.83 | May 28 | July 7 | Aug. 17 | Oct. 3 |
| *Calamagrostis rubescens* | 10.22 | 8.31 | 5.25 | 2.70 | 29.17 | 32.04 | 34.30 | 32.67 | 14.31 | 15.06 | 11.07 | 16.37 | June 17 | July 16 | Aug. 21 | Oct. 4 |

TABLE 3. (*Continued*)

| Species | Calcium | | | | Phosphorus | | | | Ca/P ratio | | | | Number of samples | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered |
| *Arnica cordifolia* | .92 | .87 | 1.48 | — | .61 | .81 | .78 | — | 1.51 | 1.18 | 1.89 | — | 2 | 3 | 2 | — |
| *Aster conspicuus* | .68 | .73 | .95 | 1.19 | .72 | .55 | .49 | .51 | .94 | 1.36 | 1.93 | 2.33 | 3 | 4 | 4 | 5 |
| *Astragalus serotinus* | .72 | .81 | .91 | — | .27 | .38 | .31 | — | 2.71 | 2.75 | 2.99 | — | 4 | 4 | 5 | — |
| *Castilleja miniata* | .82 | .96 | .95 | 2.03 | .47 | .48 | .41 | .33 | 1.74 | 1.97 | 2.29 | 6.15 | 2 | 3 | 3 | 2 |
| *Hieracium umbellatum* | — | .58 | .72 | — | — | .41 | .65 | — | — | 1.42 | 1.11 | — | — | 4 | 2 | — |
| *Lathyrus ochroleucus* | .64 | .78 | 1.16 | 1.52 | .42 | .31 | .21 | .13 | 1.52 | 2.51 | 5.52 | 11.69 | 3 | 3 | 4 | 3 |
| *Lupinus glacialis* | .82 | 1.10 | 1.31 | 1.89 | .44 | .25 | .20 | .10 | 1.86 | 4.4 | 6.55 | 18.9 | 2 | 4 | 4 | 3 |
| *Vicia americana* | .80 | .97 | 1.36 | 1.47 | .48 | .29 | .19 | .19 | 1.62 | 3.34 | 7.15 | 7.73 | 3 | 3 | 4 | 2 |
| Average | .77 | .85 | 1.10 | 1.62 | .48 | .43 | .40 | .25 | 1.70 | 2.36 | 3.67 | 9.36 | | | | |
| *Calamagrostis rubescens* | .30 | .28 | .39 | .41 | .31 | .25 | .17 | .12 | 1.14 | 1.11 | 2.32 | 3.32 | 6 | 6 | 12 | 5 |

TABLE 4. — CHEMICAL COMPOSITIONS (PERCENTAGES, OVEN-DRY BASIS) OF SEVEN FOREST SHRUBS AT VARIOUS STAGES OF GROWTH

| Species | Crude protein | | | | Crude fibre | | | | Total ash | | | | Average collection date | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered |
| *Amelanchier alnifolia* | — | 14.56 | 11.09 | — | — | 17.17 | 19.95 | — | — | 5.93 | 6.76 | — | — | June 21 | July 29 | — |
| *Rosa gymnocarpa* | 25.64 | 11.86 | 9.68 | 5.16 | 2.21 | 14.43 | 13.77 | 26.74 | 6.83 | 6.82 | 7.35 | 4.94 | May 22 | July 7 | Aug. 5 | Oct. 16 |
| *Salix barclayii* | 21.73 | 14.40 | 11.59 | 5.58 | 17.35 | 17.35 | 17.63 | 30.37 | 6.60 | 6.70 | 5.98 | 5.00 | June 17 | July 8 | Aug. 15 | Oct. 27 |
| *Shepherdia canadensis* | — | 17.81 | 13.43 | — | — | 14.13 | 16.52 | — | — | 4.48 | 4.52 | — | — | July 7 | July 24 | — |
| *Spiraea lucida* | 10.02 | 7.63 | 6.89 | 4.93 | 17.43 | 20.96 | 18.36 | 29.40 | 6.05 | 5.60 | 5.47 | 4.00 | June 17 | July 20 | Aug. 5 | Oct. 15 |
| *Vaccinium membranaceum* | — | 9.45 | 7.96 | — | — | 20.20 | 25.40 | — | — | 3.82 | 4.05 | — | — | July 18 | Aug. 12 | — |
| *Symphoricarpos albus* | 16.20 | 10.58 | 10.52 | 4.46 | 9.23 | 16.29 | 14.22 | 33.88 | 6.90 | 7.82 | 7.52 | 4.36 | May 22 | July 14 | Aug. 19 | Oct. 16 |
| Average | 18.39 | 12.32 | 10.16 | 5.03 | 14.06 | 17.21 | 17.97 | 30.09 | 6.59 | 5.88 | 5.95 | 4.57 | June 9 | July 12 | Aug. 7 | Oct. 18 |

TABLE 4. (*Continued*)

| Species | Calcium | | | | Phosphorus | | | | Ca/P ratio | | | | Number of samples | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered | Leaf | Flower | Seed | Weathered |
| *Amelanchier alnifolia* | — | .87 | 1.08 | — | — | .38 | .48 | — | — | 2.27 | 2.24 | — | — | 3 | 3 | — |
| *Rosa gymnocarpa* | .48 | 1.05 | 1.21 | .96 | .56 | .76 | .48 | .25 | .86 | 1.92 | 2.52 | 3.84 | 2 | 3 | 2 | 2 |
| *Salix barclayii* | .65 | .71 | .98 | .57 | .69 | .36 | .26 | .24 | 2.37 | 1.45 | 3.72 | 2.82 | 2 | 2 | 3 | 3 |
| *Shepherdia canadensis* | — | .35 | .48 | — | — | .17 | .19 | — | — | 2.06 | 2.53 | — | — | 2 | 3 | — |
| *Spiraea lucida* | .58 | .53 | .86 | .63 | .63 | .43 | .44 | .25 | .92 | 1.23 | 1.95 | 2.42 | 2 | 3 | 2 | 2 |
| *Symphoricarpos albus* | .33 | .68 | .86 | .50 | .48 | .29 | .42 | .17 | .69 | 2.35 | 2.04 | 3.00 | 2 | 3 | 2 | 2 |
| *Vaccinium membranaceum* | — | .68 | .68 | — | — | .17 | .19 | — | — | 4.00 | 3.58 | — | — | 2 | 3 | 2 |
| Average | .51 | .69 | .87 | .66 | .59 | .36 | .35 | .23 | 1.21 | 2.18 | 2.65 | 3.02 | | | | |

TABLE 5. — CHEMICAL COMPOSITIONS (PERCENTAGES, OVEN-DRY BASIS) OF SELECTED GRASSES AT VARIOUS STAGES OF GROWTH

| Species | Collection date | Number of samples | Crude protein | Crude fibre | Total ash | Ca | P | Ca/P ratio | Crude* fat | Nitrogen-free extract | Total* carbo-hydrates |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Agropyron inerme* | | | | | | | | | | | |
| Leaf | May 9 | 5 | 15.01 | 27.69 | 11.61 | .33 | .37 | .98 | 3.11 | 48.20 | 79.01 |
| Flower | June 15 | 8 | 8.68 | 31.87 | 8.64 | .34 | .24 | 1.44 | — | — | — |
| Seed | June 29 | 5 | 6.54 | 32.26 | 7.19 | .39 | .13 | 3.30 | — | — | — |
| Seed Shed | Aug. 10 | 8 | 4.27 | 32.47 | 10.31 | .42 | .10 | 4.20 | — | — | — |
| Weathered | Nov. 6 | 7 | 3.07 | 36.23 | 9.41 | .35 | .10 | 3.50 | 3.04 | 47.80 | 84.87 |
| *Festuca scabrella* | | | | | | | | | | | |
| Leaf | May 28 | 3 | 10.54 | 32.11 | 13.31 | .32 | .38 | .84 | — | — | — |
| Flower | July 25 | 4 | 7.27 | 33.88 | 15.16 | .35 | .32 | 1.12 | 2.45 | 45.21 | 79.97 |
| Seed | Aug. 8 | 4 | 4.83 | 37.67 | 9.86 | .30 | .19 | 1.63 | 1.22 | 45.21 | 84.12 |
| Seed Shed | Sept. 15 | 4 | 4.23 | 35.96 | 10.16 | .34 | .10 | 3.00 | — | — | — |
| Weathered | Nov. 25 | 3 | 2.05 | 42.47 | 12.27 | .28 | .08 | 3.39 | 3.00 | 46.64 | 82.60 |

*Figures based on one sample from each of three locations

TABLE 5. (*Continued*)

| Species | Collection date | Number of samples | Crude protein | Crude fibre | Total ash | Ca | P | Ca/P ratio | Crude* fat | Nitrogen-free extract | Total* carbo-hydrates |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Calamagrostis rubescens* | | | | | | | | | | | |
| Leaf | June 17 | 6 | 10.22 | 29.17 | 14.31 | .30 | .31 | 1.14 | — | — | — |
| Flower | July 16 | 6 | 8.31 | 32.04 | 15.06 | .28 | .25 | 1.11 | 1.81 | 44.08 | 73.78 |
| Early seed | Aug. 5 | 6 | 5.81 | 33.27 | 10.76 | .38 | .17 | 2.17 | 2.10 | 40.04 | 71.11 |
| Late seed | Sept. 2 | 6 | 4.68 | 35.32 | 11.31 | .39 | .15 | 2.47 | 1.74 | 40.51 | 70.83 |
| Weathered | Oct. 3 | 5 | 2.70 | 32.67 | 16.37 | .41 | .12 | 3.32 | — | — | — |
| *Calamagrostis rubescens* 60%, Mixed forbs 40% | | | | | | | | | | | |
| | July 6 | 2 | 10.60 | 26.75 | 14.54 | .71 | .27 | 2.63 | 2.77 | 45.33 | — |
| | July 28 | 2 | 9.58 | 26.72 | 17.96 | .76 | .27 | 2.81 | 1.61 | 44.12 | — |
| | Sept. 22 | 2 | 4.06 | 30.76 | 18.01 | .74 | .15 | 4.93 | 2.29 | 44.87 | — |

TABLE 6. — CHEMICAL COMPOSITIONS (PERCENTAGES, OVEN-DRY BASIS) OF A TYPICAL HIGH ELEVATION SEDGE MEADOW NEAR KAMLOOPS, B.C. (AVERAGE OF FOUR SAMPLES)

| Treatment | Date | Crude protein | Crude fibre | Total ash | Calcium | Phosphorus | Ca/P ratio |
|---|---|---|---|---|---|---|---|
| Clipped every 2 weeks | June 27 | 13.33 | 27.01 | 7.45 | 0.39 | 0.16 | 2.44 |
| | July 9 | 13.01 | 29.48 | — | — | — | — |
| | Aug. 6 | 15.78 | 24.68 | — | — | — | — |
| | Sept. 3 | 14.05 | 23.83 | — | — | — | — |
| | Sept. 17 | 9.49 | 27.32 | 10.13 | 0.48 | 0.11 | 4.36 |
| Clipped every 4 weeks | June 27 | 13.18 | 27.83 | 7.48 | 0.39 | 0.15 | 2.60 |
| | July 23 | 15.09 | 25.38 | — | — | — | — |
| | Aug. 21 | 14.76 | 23.78 | — | — | — | — |
| | Sept. 17 | 9.31 | 27.35 | 10.86 | 0.42 | 0.10 | 4.20 |
| Clipped every 4 weeks starting July 23 | July 23 | 11.07 | 24.89 | 8.32 | 0.54 | 0.13 | 4.15 |
| | Aug. 21 | 10.46 | 25.72 | — | — | — | — |
| | Sept. 17 | 7.56 | 27.72 | 10.16 | 0.48 | 0.095 | 5.33 |
| Clipped at end of season only | Sept. 17 | 6.46 | 28.24 | 9.86 | 0.58 | 0.07 | 8.28 |

TABLE 7. — CHEMICAL COMPOSITIONS (PERCENTAGES, OVEN-DRY BASIS) OF MIXED NATIVE MEADOW HAY AND OF SINGLE SPECIES FROM STACKS AT VARIOUS LOCALITIES (AVERAGE OF THREE SAMPLES)

| Botanical composition | Locality | Crude protein | Crude fibre | Total ash | Ca | P | Ca/P |
|---|---|---|---|---|---|---|---|
| Mixed sedge hay | Kamloops | 9.59 | 21.71 | — | 1.20 | .14 | 8.57 |
| Mixed sedge hay | Tranquille | 9.01 | 27.96 | 7.37 | 1.38 | .45 | 3.07 |
| Mixed sedge hay | Lower Cariboo | 8.79 | 28.84 | 5.23 | .34 | .08 | 4.25 |
| *Agrostis stolonifera* | Kamloops | 5.29 | 28.84 | — | .30 | .10 | 2.98 |
| *Carex atherodes* | Kamloops | 5.77 | 27.50 | — | .62 | .08 | 7.94 |

Watkins (16) found that the phosphorus content of grasses on New Mexico grassland ranges met the requirements of cattle for only a short period during the grazing season. On the other hand, Stoddart and Greaves (11) found no deficiency of phosphorus or other minerals in plants on mountainous summer ranges where the forage was consumed while still green and relatively immature. From early August until the close of the grazing season, progressively smaller amounts of protein and phosphorus are present in the forage while the amount of the crude fibre increases and the digestibility decreases. The percentage of total digestible nutrients probably declines also. The rapid drop in chemical composition of the forest range in the late summer suggests the value of an early move from this type of range for cattle destined for market that year. In addition to the changes in chemical composition occurring during seasonal growth development, further declines of certain nutrients, particularly protein and phosphorus, have been reported in forage exposed to rain and snow over winter (7, 2).

The interpretation of results from fall range forage is complicated by the effects of selective grazing on parts of individual plants and the availability of green fall growth in many years.

Cook *et al.* (6) found that chemical analyses of entire plants and of

parts commonly grazed showed that livestock selected the leafier and more nutritious portions. Sheep were more selective than cattle in this regard. Both kinds of livestock obtained a diet that at any given period was higher in protein, phosphorus, and gross energy than was total current growth. This selectivity became more pronounced as plant maturity increased. Separate chemical analyses of leafage and stems in range forages have shown considerable differences in composition. Cook and Harris (4), working with grasses, forbs, and browse, found that leafage was usually higher in protein, ether extract, phosphorus, calcium, and nitrogen-free extract, whereas stems were higher in crude fibre, lignin, and cellulose. Clarke and Tisdale (2) found a similar situation in grasses of the Canadian Great Plains.

If poor hay is fed, deficiencies are not likely to be offset by eating weathered grass for this class of feed is low in both protein and minerals. Grasses, however, are good sources of energy for winter maintenance. Supplemental feeding of calves on fall and winter range may particularly be suggested since this forage is generally low in protein and energy, the levels being generally adequate to maintain body weight but inadequate for the growth desired in young animals. Benefits resulting from the feeding of mineral supplements, including phosphorus, have been observed frequently on local ranches.

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# BREEDING FOR CREEPING ROOT IN ALFALFA (*MEDICAGO MEDIA* PERS.)[1]

F. H. W. MORLEY[2] AND D. H. HEINRICHS[3]

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## ABSTRACT

An alfalfa population, consisting of progenies from crosses among 54 plants which had been selected out of a large population of *Medicago falcata* L. x *Medicago media* Pers. origin, was scored for degree of creeping-root development. These scores were statistically analysed on the basis of full-sib and half-sib groups. The estimate of Cov(FS) — Cov(HS) was 0.062, and Cov(HS) was 0.091. Therefore, it was concluded that genotypic variation was predominantly additive.

Mass selection alone is predicted to be more efficient in breeding for the creeping-rooted character in alfalfa than a combination of mass selection and progeny testing. However, since other characters will usually be selected for along with the creeping-rooted character, the progeny test may have to be used. If progeny testing is used, the optimum number of progeny per parent under test should be approximately 30, provided these have been obtained from at least 8 to 10 different, unrelated, and randomly-selected mates.


## INTRODUCTION

A description of the creeping-rooted character in alfalfa and its potential agronomic value has been presented by Heinrichs (2) and Murray (8). Recently the variety Rambler, within which 65 per cent of the plants are creeping rooted, has been released in Canada and is at present undergoing extensive tests (3).

Alfalfa breeders in many countries are keenly interested in the possibility of incorporating the creeping-rooted character into strains adapted to local conditions. It is, therefore, of wide interest to obtain information on the inheritance of this trait, and to use the information in the design and evaluation of breeding programs.

In this paper, data obtained on the creeping-rooted character from a large population of alfalfa grown in experimental blocks at Swift Current, Saskatchewan, have been statistically analysed with the object of determining whether genotypic variance in the population was predominantly additive or included non-additive components. If non-additive genotypic variance should be important, special techniques of selection, which make use of specific combining ability, would be necessary. However, if additive genotypic variance is large relative to other sources of variance, mass selection and other techniques of selection for general combining ability would be preferred. The relative emphasis to be placed on performance, progeny testing or other aids to selection depends on resources demanded by various breeding plans as well as on the different components of variance.

On the basis of the results obtained several breeding plans were examined with the object of demonstrating some of the principles used in applying the results of the analysis. It is possible that the results obtained in this study may not be entirely applicable to programs with other breeding

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stock or in different environments. The results, therefore, should be regarded as a first approximation until further evidence provides a basis for future modification.

## MATERIALS AND METHODS

The alfalfa population under study, consisting of 2,967 plants, has been described by Heinrichs (2) whose Table 1 gives a sample of the data. It consisted of progenies from crosses among 54 plants (of a total of 58 available) which were selected from a large population of *Medicago falcata* L. x *Medicago media* Pers. origin. An occasional cross line was omitted from the analysis because there were insufficient plants or because it could not be conveniently grouped.

The data analysed were the scores for creeping root taken in the spring of 1950 when plants were approximately 2 years old. The plants were scored 1, 2, 3, 4, or 5, depending on the amount of creep development (1 = strongly creeping rooted with a spread of 36 inches in diameter or more; 5 = the other extreme with no creeping-rooted tendency in evidence; the other 3 scores represent degrees of creeping-root development between these extremes).

The field experiment was planted in 1948. The progeny of each cross were divided into two lots, one being planted in each of two replicates with positions assigned at random. The plants in a progeny group within a replicate were space planted adjacent to each other in a row.

The crosses were arranged in 34 groups, each group being the progeny of one plant crossed with a number of unrelated plants. The genetical relationships among plants were:

(1) Full sibs where both parents were in common
(2) Half sibs where one parent was in common
(3) Zero relationship otherwise.

The actual relationship between groups was not quite zero since in several cases groups would include one or more parents in common. Thus, plant A might be mated with B, C, D, and E, and plant K with B, F, G, H, and I. If $k$ = number of crosses in each of two randomly chosen half-sib groups, and $m$ = the number of crosses in one group with one parent in common with a cross in the other group, the average covariance among groups would be $\mathrm{Cov(HS)}\ m/k^2$. Since $m$ was usually zero and seldom greater than 1 and $k$ was approximately 5, this procedure introduced a bias which would be negligible relative to other sources of statistical error.

This experimental design and genetical structure resembles that described by Kempthorne (5, Section 20.8), and the same notation will be used in the following argument, describing the common parent in a group as the sire and the variable parents as the dams. Kempthorne's population consisted of $s$ sires, each mated to a random sample of $d$ dams, the progeny of each cross being tested in $r$ replicates of a randomized block experiment. The number of individuals measured from the cross of sire $i$ with dam $ij$ in replicate $k$, was given as $N\mathrm{ijk}_{,,}$ a number greater than zero for all $i$, $j$, and $k$.

## RESULTS

The modification of Kempthorne's analysis and estimation procedures made necessary because $d$ varied from sire to sire, and which takes into account a possible genotype x replicate interaction, has been presented by Morley (7). The modified analysis of means, and expectations of mean squares, are given in Table 1, using Kempthorne's notations in which

$\sigma_1^2$ = variance among individuals within a plot, that is, within plot environmental variance plus total genotypic variance less the covariance among full sibs (Cov(FS)).

$\sigma_e^2$ = the variance due to plot effects plus the interaction of cross x replicate.

Cov(HS) = the covariance among half sibs.

$\sigma_{sr}^2$ = the interaction of sire group x replicate.

$\sigma_r^2$ = variance among replicates.

Equating the mean squares of Table 1 to their expectations, the following estimates were obtained:

$\sigma_1^2 = 1.342$

$\sigma_{sr}^2 = (0.203 - 0.184)/4.546 = 0.004$ (n.s.)

$\sigma_e^2 = 0.028$ (n.s.)

$\text{Cov(FS)} - \text{Cov(HS)} = (0.307 - 0.184)/2 = 0.062$

$\text{Cov(HS)} = (1.149 - 0.307 + 0.184 - 0.203)/9.091 = 0.091$

In diploids the quantity Cov(FS) − 2 Cov(HS) provides an indication of the presence of non-additive genotypic variation. As this quantity is negative we may infer that the genotypic variation is predominantly additive. But since inheritance in alfalfa may be tetraploid, the diploid model may be inappropriate.

Following Kempthorne (4, and 5, Table 18.1) the genotypic variance in a one-locus auto-tetraploid population in which segregation is chromosomal may be due to four kinds of genetic effect, which he termed average

TABLE 1. — ANALYSIS OF VARIANCE OF MEAN SCORES

| Source | d.f. | M.S. | E.[M.S.] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | $\sigma_1^2$ | $\sigma_e^2$ | Cov(HS) | Cov(FS)-Cov(HS) | $\sigma_{rs}^2$ | $\sigma_r^2$ |
| Replicates | 1 | 0.200 | 0.116 | 1 | 0 | 0 | 4.806 | 310 |
| Sires (Common Parents) | 33 | 1.149*** | 0.116 | 1 | 9.091 | 2 | 4.546 | 0 |
| Dams (Variable Parents) in Sires | 121 | 0.307** | 0.116 | 1 | 0 | 2 | 0 | 0 |
| Sire x Replicates | 33 | 0.203 | 0.116 | 1 | 0 | 0 | 4.546 | 0 |
| Residual | 121 | 0.184 | 0.116 | 1 | 0 | 0 | 0 | 0 |
| Within Plot* | 2657 | 1.342 | 1.000 | 0 | 0 | 0 | 0 | 0 |

*From a separate analysis (following Kempthorne)
**P. less than 0.01
***P. less than 0.001 [Satterthwaite's (10) approximation]

gene effects, viz., additive (A), dominance deviations (D), trigenic deviations (T), and quadrigenic deviations (F). In this paper we need to estimate the parent-offspring regression in order to study the effect on progress of various systems of selection. Using Kempthorne's definitions the following identities may be set up:

$$\text{Cov Full Sib} = \frac{1}{2}A + \frac{2}{9}D + \frac{1}{12}T + \frac{1}{36}F = \text{Cov(FS)}$$

$$\text{Cov Half Sib} = \frac{1}{4}A + \frac{1}{108}D = \text{Cov(HS)}$$

$$\text{Cov Parent-Offspring} = \frac{1}{2}A + \frac{1}{6}D = \text{Cov(PO)}$$

2 Cov(HS) thus provides a reasonable approximation of Cov(PO) unless dominance deviations are large. The identity Cov(FS) – 2 Cov(HS) provides an indication of the size of non-additive deviations, which in this case must be approximately zero. We may, therefore, in subsequent calculations use the relationship G = 2 Cov(FS) = 2 Cov(PO) = 4 Cov (HS) where G = genotypic variance (in this case all additive). The possibility exists that this relationship may change as selection changes the population, but the history of selection experiments suggests that extrapolation for five generations would not be unjustifiably venturesome.

## APPLICATION OF RESULTS

The results of various kinds of selection may be predicted by using the theory developed by Dickerson and Hazel (1) and other students of animal breeding.

In material of this nature we may use mass selection alone or in combination with aids such as progeny testing. The annual improvement expected from any type of selection (9) may be written as

$$\Delta g = \sum_j s\, b_j\, \sigma x_j / \sum_j L_j$$

where $\Delta g$ = the change in genotype per annum

$s_j$ = the selection differential in standard deviations of $x_j$ (calculated in the case of truncation selection of a normally distributed population as $3/p$ where $p$ is the proportion selected, and $z$ the height of the ordinate at the point of truncation), assuming normal distribution and truncation selection.

$b_j$ = the regression of breeding value of the selected individual on the index of selection such as the phenotype of the plant or the mean of a progeny group.

$\sigma x_j$ = the standard deviation of the index.

$L_j$ = the number of years from seed of one generation to seed of the next generation, that is, the generation length.

As an example we might consider a population of about 5,000 plants in which a combination of mass selection and progeny testing is to be applied. The procedure might be along the following lines:

*Year 1, Spring* – Plant 5,000 plants in the field.

*Year 2, Spring* – Score plants for degree of creep, select the best 100. Make a number of propagules of these and plant in a polycross block for seed production.

*Year 2, Autumn* – Harvest seed from the polycross block.

*Year 3, Spring* – Plant out progenies in a randomized block test, 50 plants per progeny.

*Year 4, Spring* – Score progenies, select the best 20 lines (cutting other propagules out of crossing block).

*Year 4, Autumn* – Harvest seed from elite crossing block for planting next spring. The cycles may then be repeated.

The proportion selected in the spring of Year 2 would be $100/5000 = 0.02$. The selection differential $S_1$, calculated from a table of the normal curve, would, therefore, be 2.415 standard deviations.

The regression, $b_1$, of the transmitting ability of the parent on its own score is $G/\sigma^2$, where $\sigma^2$ is the phenotypic variance in the original population. Taking as an estimate of G a function of the estimates of Cov(HS) and Cov(FS), $G = 2(0.091 + 0.062) = 0.306$.

The appropriate value for $\sigma^2$ will depend somewhat on experimental layout. Assuming that all the sources of error within replicates are applicable we use the estimate

$$\sigma^2 = \sigma_1^2 + \sigma_e^2 + \sigma_{ie}^2 + \tfrac{1}{2}G$$

$b_1$ is therefore

$$\begin{aligned} &2 \times 0.153/(1.342 + 0.028 + 0.004 + 0.153) \\ &= 0.306/1.527 \\ &= 0.20 \end{aligned}$$

The first item in the numerator of $\Delta g$ is, then,

$$\begin{aligned} S_1 b_1 &= 2.42 \times 0.20 \times 1.236 \\ &= 0.598 = 0.484\sigma \end{aligned}$$

In calculating the effect of selection based on progeny tests allowance must be made for the reduction in genotypic variance caused by the initial selection. A modification of a formula proposed by Cochran (1) is

$$G_s/G_o = 1 - G/\sigma^2 [S(s-t)]$$

where $G_s$ is the variance remaining after the selection coefficient S has been obtained by truncation selection at $t$ standard deviations from the mean.

In this example:

$$G_s/G_o = 1 - 0.20 \times 2.415\,(2.415 - 2.055) = 0.83$$

that is

$$G_s = 0.83 \times 0.306 = 0.254$$

The values for $t$ may be obtained from tables of areas and ordinates of the normal curve.

The progeny group obtained from the crossing block will consist of plants derived from a number of pollen parents, but only one seed parent in each case. Let there be $p$ progeny from each of $m$ mates providing pollen. Let the score of the $k^{th}$ offspring of the $j^{th}$ mate of the $i^{th}$ plant under test be designated $O_{ijk}$.

The regression of parent genotype on the mean of the progeny group is the covariance of the parent genotype and offspring mean divided by the variance of offspring means ($V_o$). The numerator is clearly $Cov(PO) = G_s/2$. The denominator may be derived from the following

model: Let $O_{ijk} = \mu + g_{ijk} + e_{ijk}$ where $g_{ijk}$ represents the genotypic value and e the environmental effect.

Let $E[g_{ijk}^2] = G_s$

$E[e_{ijk}^2] = E$

$E[O_{ijk} \cdot O_{ijk'}] = \text{Cov(FS)} = G_s/2$

$E[O_{ijk} \cdot O_{ij'k'}] = \text{Cov(HS)} = G_s/4$

$E[O_{ijk} : O_{i'j'k'}] = 0$

$$E\left[\frac{(O_i \cdot - mpu)^2}{mp}\right] = V_o$$

$$= \frac{1}{m^2p^2}[\Sigma_{jk}(g_{ijk} + e_{ijk})]^2$$

$$= \frac{1}{m^2p^2}[mp(G_s + E) + mp(p-1)\text{Cov(FS)} + mp^2(m-1)\text{Cov(HS)}]$$

$$= \frac{1}{4mp}[E + G_s(p(m+1)+2)]$$

The improvement resulting from the second stage of selection, $S_2 b_2 \sigma x_2$ is thus calculated to be $S_2$

$$\frac{G_s}{2}\ \frac{\sqrt{V_o}}{V_o}$$

$$= \frac{S_2\, G_s\sqrt{mp}}{\sqrt{E + G_s\,[p(m+1)+2]}}$$

$$= \frac{S_2 \times 0.254\,\sqrt{mp}}{\sqrt{1.221 + 0.254\,[p(m+1)+2]}}$$

Since 20 plants are to be selected from the 100 progeny-tested, $S_2 = 1.40$.

The improvement is thus expected to be

$$\frac{1.40 \times 0.254\,\sqrt{mp}}{\sqrt{1.729 + 0.254\,[p(m+1)]}}$$

$= 0.634$ or $0.512\,\sigma$ if $m = 10$ and $p = 5$.

The total improvement from the two stages of selection is then $(0.484 + 0.512)\sigma$.

Since the cycle takes 4 years the annual improvement is $0.249\sigma$.

The effect of varying $m$ and $p$ may be calculated, with results shown in Figure 1 for $mp = 48$.

The rate of improvement is near maximum if 8 to 12 mates are used but falls off fairly steeply with fewer mates than this. Since the number of mates crossing with each propagule in a crossing block is likely to be at least 4, there seems little point in having more than 4 to 5 replicates, provided crossing is not restricted by variation in flowering time or other causes, so that only few crosses are successful.

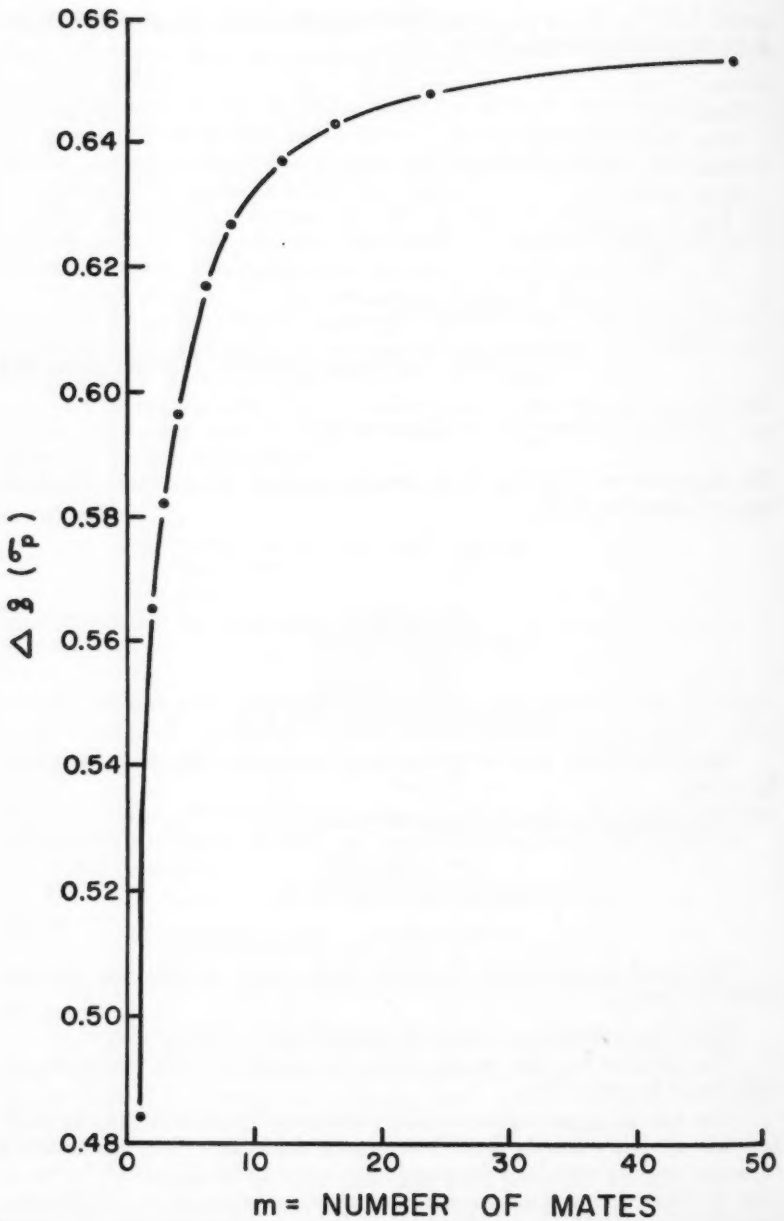


FIGURE 1. Effect of number of mates on efficiency of selection by progeny tests.

The improvement from mass selection, alone or combined with progeny-testing, was predicted by using various formulae derived above, assuming always that $p$, the number of progeny per mate, was one. As can be seen from Figure 1 this result would not be affected greatly if $p$ were greater than 1, provided $m$ were greater than 6 to 8. The results of this examination are shown graphically in Figure 2.

The curves in Figure 2A represent the predicted annual improvement in two hypothetical populations, one of 5,000 plants, the other of 2,500 plants. These show that mass selection alone should give faster progress than mass selection combined with progeny testing, if the ratio $G/\sigma^2$ is 0.2. The effect of this ratio being only 0.1 was examined in Figure 2B. Here a slight improvement is brought about by the use of progeny testing but the advantage might not be sufficient to offset the effort involved.

If progeny tests are to be used, there is little point in having more than 30 progeny per parent plant tested. In that respect the material of this study was not the most efficient design, there being generally more than 100 progeny per parent. The optimum number of progeny per plant tested seems to depend more on population size than on the ratio $G/\sigma^2$. In the smaller population the intensity of selection in the first stage would be less, there would be more genotypic variation among selected individuals, and hence fewer progeny would be required to select in the second stage.

In this section a fully randomized arrangement of plants has been assumed, with some device for removing replicate variance (although this was negligible in the present results). If progeny tests are set out in plots, as was done with this material, some loss of efficiency will result since the number of replications must be reduced, the replicates made larger, and comparisons less precise. In the present example replicate variation, and variation among plots within replicates, were both very small compared with genotypic differences. Therefore, the design of a test would affect precision to only a negligible extent. However, this situation may not always prevail, and the number of replicates should generally be the maximum consistent with simplicity in planting, maintenance, and recording of results.

No attempt has been made to evaluate the use of clonal testing or techniques such as that of Smith (11) of culturing root cuttings by special greenhouse techniques. A full evaluation of these must await further clarification of the correlation between such tests and field performance. Clearly any technique which shortens the generation interval is likely to be useful, but how useful cannot yet be determined.

As the level of expression increases in response to selection, progress may be increasingly hampered by the inability to discriminate between degrees of creeping. At such a stage the efficiency of selection may be aided by root culture, and also by progeny testing. Progeny testing may be especially valuable in selecting for other desired characteristics, especially if heritability is low.

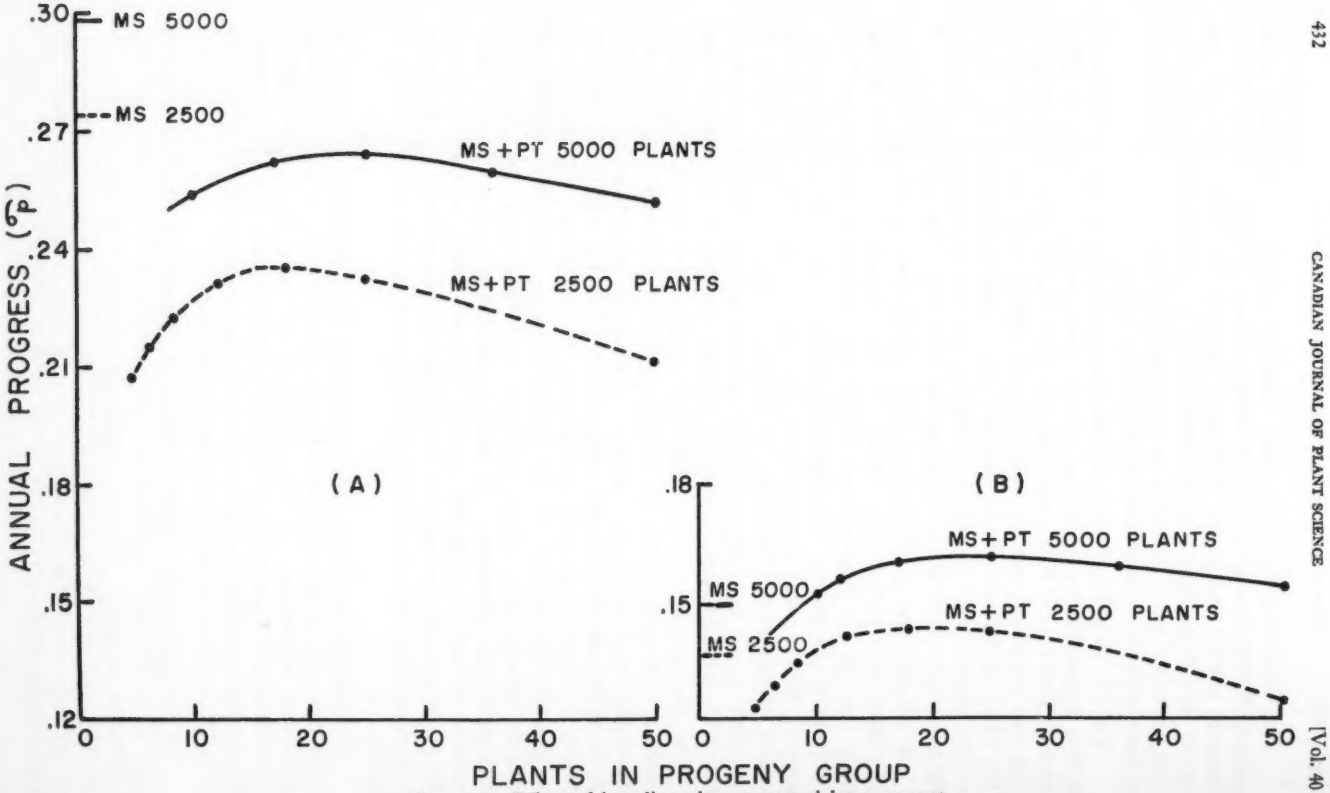


FIGURE 2. Effect of breeding plan on annual improvement.

## CONCLUSIONS

The genotypic variation in degree of creeping-rootedness is predominantly additive in the population studied, but was only about 20 per cent of the total phenotypic variance. Mass selection is predicted to be preferable to a combination of mass selection and progeny testing, as judged by annual improvement from selection. However, the combination may be preferred because of information required on other characters, and because the size of the genotypic variance may be continually checked.

If progeny testing is used the optimum size of progeny is approximately 30 plants, provided these are obtained by crossing with at least 8 to 10 randomly selected mates. However, considerable departures from this optimum are possible without substantial loss in efficiency.

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# FERTILITY OF AUTOTETRAPLOID VARIETIES AND HYBRIDS OF BARLEY[1]

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## ABSTRACT

This study was conducted from 1952 to 1958. Most of the autotetraploids were obtained by immersing germinated diploid seed in a 0.1 per cent aqueous solution of colchicine for 2 hours. Selection for fertility within the progenies of the $C_3$ and $C_4$ generation of autotetraploid hybrids increased the mean per cent fertility of the $C_4$ generation by 8 per cent and the $C_5$ by 4 per cent. Fertility studies of artificially-induced autotetraploids of several barley varieties showed that two-rowed autotetraploids were more fertile than six-rowed types. In all cases the fertility of autotetraploids was lower than diploids.

## INTRODUCTION

Selection has improved the fertility in autotetraploids[3] of cross-fertilizing crops such as rye, buckwheat, rape, red clover, and alsike clover (1, 2) to allow for commercial production, but not in self-fertilizing crops such as barley.

Müntzing (4) reported that the first tetraploid strain of barley produced at Svalöf is still, after 20 years, just as poor and sterile as in the first years of cultivation. He stated that seldom in self-fertilizing crops is there an increase in fertility by selection without recombination. Reinbergs and Shebeski (8) were unable to change the mean per cent fertility level of tetraploid barley variety O.A.C. after four generations of selection. They postulated that selection for increased fertility should be made in heterogeneous progeny of tetraploid hybrids. Ono (5, 6, 7) believes that successive selection in homozygous varieties would increase their fertility since he was able to increase the fertility of tetraploid varieties after seven generations of selection.

The purpose of this investigation was to compare the fertility of tetraploids derived from intervarietal crosses and to determine the effect of selection for fertility in these tetraploids.

## MATERIALS AND METHODS

Four varieties of barley, differing widely in geographic origin and phenotypic characters, were diallel crossed. The varieties used were *Awnless* (an introduced spring variety from Russia); *Herta* (an introduced spring variety from Sweden); *40 Day* (an introduced hulless, spring variety from Afghanistan), and *Wong* (an introduced winter variety from China).

The crosses were made in the greenhouse during the winter of 1952, (January and April), and the $F_1$ and parental diploid seeds were subsequently subjected to various colchicine treatments during the summer of 1952. The treatment that was found to be most effective in producing a high proportion of tetraploids (20 per cent of seeds treated) was as follows: Barley seeds were germinated for 48 hours in Petri dishes containing moist sand. The seedlings were then immersed for 2 hours in a 0.1 per cent aqueous solution of colchicine. After treatment the seedlings were rinsed in water and sown in a bed in the greenhouse or outdoors.

[1]Part of a Ph.D. thesis submitted to the Graduate College, University of Nebraska.
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[3]Hereafter referred to as "tetraploids".

The $C_1$ generation hybrids and parental plants were pulled in the fall of 1952 and each head was harvested separately. Counts were made of the total number of seeds planted and the number of plants that survived to maturity. Attempts were made to identify tetraploid plants in this material by pollen and stomata size without success.

In the spring of 1953 the seed from each of the heads of the $C_1$ plants was sown in a separate 5-foot headrow and examined morphologically for tetraploids.

Spikes of secondary tillers from $C_2$ and $C_3$ putative tetraploid plants were collected and stored in Carnoy's solution for cytological identification.

Fertility classifications were made by selecting one mature head at random from each plant, and counting the total number of central florets and the total number of seeds set in them. Previous tests had shown that there was a high correlation ($r = 0.75$) between the mean fertility of all spikes produced by a plant and a single randomly selected spike. The per cent fertility was determined by calculating the proportion of central florets which produced seed.

The $C_2$ tetraploids derived from diploid hybrid plants were threshed individually and those which had produced enough seed to sow a rod-row were selected for field planting in 1956. The $C_3$ rod-rows were examined and classified for tetraploidy in the field on the basis of morphological characters. Cytological classification was also made by examining pollen mother cells. The pedigree system of propagating lines individually was used throughout the study, so that every plant in advanced generations could be traced to its original parent plant.

Each $C_3$ tetraploid plant, in rows classified as tetraploids, was harvested separately and classified for fertility. Selections for fertility were made at three levels: high fertility (above 75 per cent), medium fertility (exactly 50 per cent), and high sterility (below 25 per cent).

A single head from each $C_3$ tetraploid row was chosen at random and multiplied for two generations by selfing in the greenhouse, during the winter of 1956-57, to obtain $C_3$, $C_4$ and $C_5$ seed for field testing in 1957.

Head-progenies from the selected $C_3$ heads, their $C_4$ and $C_5$ progenies, and the fertility selections from the $C_3$ progenies, were sown in head-hills in the spring of 1957.

A second cycle of selection for fertility was made from the $C_4$ progenies of $C_3$ selections and sown in head-rows in 1958. The high, medium and low fertility selections were taken from the progenies of the previously selected corresponding $C_3$ fertility classes.

In 1956 Colchicine-induced tetraploids were produced from two-rowed barley varieties Jet and Sanalta; and six-rowed varieties Vantage, Gateway, Montcalm, Wolfe, and O.A.C. 21. In addition, $C_{12}$ tetraploid seed of a Japanese two-rowed variety Saitama was obtained from Dr. Ono.

The eight tetraploid varieties produced in 1956, plus the two tetraploid varieties, Herta and 40 Day, obtained from previous colchicine treatments, were sown in a randomized block arrangement with six replicates in the greenhouse during the winter of 1956-57 to compare the fertility of two-rowed and six-rowed tetraploids.

The ten tetraploid varieties, together with $C_4$ seed of selections, from 40 Day x Herta, and Wong x Herta, were sown in the greenhouse in the spring of 1957 for crossing purposes. The $F_1$ seed from hybrids between tetraploids was sown during the summer of 1957 and observations were made on survival and general vigour. Fertility classification was not obtained on the $F_1$ material due to bird damage. The $F_2$ progenies of these hybrids were sown in the greenhouse in March, 1958, and fertility classifications were made in June of that year. High and low fertility selections of the earliest hybrids were made from the $F_3$ progenies of these hybrids and sown in the field in 1958 for fertility studies under field conditions.

Seed of the $C_2$, $C_3$ and $C_4$ generations of 8 varieties (Jet, Sanalta, Herta, 40 Day, Montcalm, Wolfe, O.A.C. 21, and Vantage) was sown in the field in 1957 to make fertility comparisons under field conditions within these unselected generations from tetraploids of homozygous varieties.

## EXPERIMENTAL RESULTS

A high proportion of treated $C_1$ plants showed evidence of physiological disturbances. Some plants had one or more unusually broad leaves when compared to normals. Also, a few spikes showed considerable sterility, indicating the possibility of a doubled sector. Such heads, however, rarely produced tetraploid progeny in the $C_2$.

Observation of the $C_2$ generation indicated that the main effect of the colchicine treatments was the production of chimeras. Of the 79 $C_1$ plants which produced tetraploid progeny only 4 were completely tetraploid, the remaining 75 were mixoploids. The tetraploid plants in the

TABLE 1.—MEAN FERTILITY PERCENTAGES (WITH STANDARD ERRORS) OF UNSELECTED TETRAPLOID PROGENIES WITHIN VARIETIES AND HYBRIDS STUDIED IN SIX SUCCESSIVE GENERATIONS ($C_2$-$C_7$ FROM 1952 TO 1958)

| Variety or hybrid | $C_2$ | $C_3$ | $C_4$ | $C_5$ | $C_6$ | $C_7$ |
|---|---|---|---|---|---|---|
| 40 Day | 10±2 | 13±4 | 28±13 | 30±9 | 30±7 | 26±4 |
| Herta | 50±5 | 47±3 | 49±3 | 69±6 | 56±4 | 61±9 |
| Awnless x 40 Day | 55±1 | 50±1 | 59±2 | 53±2 | 54±2 | 38±2 |
| Awnless x Herta | 65±4 | 60±1 | 65±2 | 60±4 | 61±5 | 66±2 |
| 40 Day x Herta | 54±1 | 46±1 | 52±4 | 53±3 | 53±6 | 40±2 |
| Wong x Herta | 62±1 | 56±1 | 71±2 | 66±3 | 62±2 | 60±2 |
| Wong x 40 Day | 56±3 | 51±1 | 66±2 | 55±4 | 60±3 | 58±1 |
| Means | 50 | 46 | 56 | 55 | 54 | 50 |

TABLE 2. — COMPARISONS OF THE DISTRIBUTIONS OF FERTILITY IN THE PROGENIES OF $C_3$ PLANTS OF DIFFERENT FERTILITIES

| Variety or hybrid | $C_3$ fertility "above 75%" | | | $C_3$ fertility "50%" | | | $C_3$ fertility "below 25%" | | | $X_i^2$[1] |
|---|---|---|---|---|---|---|---|---|---|---|
| | $C_4$ fertility classes (%) | | | $C_4$ fertility classes (%) | | | $C_4$ fertility classes (%) | | | |
| | 0–29 | 30–59 | 60–100 | 0–29 | 30–59 | 60–100 | 0–29 | 30–59 | 60–100 | |
| Herta | 1 (7)[2] | 6 (43) | 7 (50) | 0 (0) | 6 (60) | 4 (40) | 2 (33) | 3 (50) | 1 (17) | 6 |
| Awnless x 40 Day | 5 (3) | 58 (32) | 119 (65) | 6 (32) | 59 (31) | 123 (67) | 18 (13) | 49 (36) | 68 (51) | 22** |
| Awnless x Herta | 0 (0) | 15 (21) | 57 (79) | 1 (2) | 11 (20) | 42 (78) | 5 (12) | 15 (37) | 21 (51) | 17** |
| 40 Day x Herta | 5 (4) | 53 (45) | 60 (51) | 2 (3) | 31 (49) | 30 (48) | 23 (30) | 37 (50) | 15 (20) | 45** |
| Wong x Herta | 1 (.3) | 48 (13) | 323 (87) | 2 (1) | 53 (34) | 102 (65) | 11 (8) | 49 (36) | 77 (56) | 78** |
| Wong x 40 Day | 10 (5) | 29 (14) | 170 (81) | 8 (5) | 48 (33) | 90 (62) | 21 (22) | 33 (35) | 40 (43) | 72** |
| $X_i^2$ | 87** | | | 37** | | | 41** | | | |

[1] $X_i^2$ – Chi-square for independence

[2] Numbers in parentheses are percentages.

TABLE 3.—MEAN PER CENT FERTILITY OF $C_4$ GENERATION TETRAPLOID BARLEY PLANTS DERIVED FROM THREE DIFFERENT FERTILITY CLASSES SELECTED IN THE $C_3$ GENERATION GROWN IN 1957

| Variety or hybrid | $C_3$ "above 75%" | | $C_3$ "50%" | | $C_3$ "below 25%" | |
|---|---|---|---|---|---|---|
| | $C_4$ mean | No. of plants tested | $C_4$ mean | No. of plants tested | $C_4$ mean | No. of plants tested |
| 40 Day[1] | — | | — | | 31±8 | 6 |
| Herta | 51±8 | 14 | 60±5 | 10 | 45±9 | 6 |
| Awnless x 40 Day | 63±1 | 182 | 64±1 | 188 | 55±2 | 135 |
| Awnless x Herta | 71±4 | 72 | 68±3 | 54 | 55±3 | 41 |
| 40 Day x Herta | 56±3 | 118 | 56±3 | 63 | 44±3 | 75 |
| Wong x Herta | 72±1 | 372 | 67±1 | 157 | 58±2 | 137 |
| Wong x 40 Day | 71±2 | 209 | 62±2 | 144 | 55±3 | 94 |
| Means | 64 | | 63 | | 49 | |

[1]Could not select for high or medium fertility

TABLE 4.—MEAN PER CENT FERTILITY OF $C_5$ GENERATION TETRAPLOID BARLEY PLANTS DERIVED FROM THREE FERTILITY CLASSES SELECTED IN THE $C_4$ GENERATION GROWN IN 1958

| Hybrid | $C_4$ "above 75%" | | $C_4$ "50%" | | $C_4$ "below 25%" | |
|---|---|---|---|---|---|---|
| | $C_5$ mean | No. of plants tested | $C_5$ mean | No. of plants tested | $C_5$ mean | No. of plants tested |
| Awnless x 40 Day | 42±2 | 123 | 49±2 | 186 | 39±1 | 138 |
| Awnless x Herta | 62±2 | 92 | 63±2 | 53 | 51±2 | 12 |
| 40 Day x Herta | 53±3 | 66 | 43±3 | 25 | 34±1 | 74 |
| Wong x Herta | 61±1 | 559 | 44±2 | 177 | 60±1 | 520 |
| Wong x 40 Day | 57±2 | 425 | 43±2 | 70 | 42±1 | 138 |
| Means | 55 | | 48 | | 45 | |

$C_2$ generation were easily distinguished by their very erect growth habit, short straw, and reduced fertility. Cytological examinations of plants from each $C_2$ head-row with putative tetraploids revealed that no diploid plants were present.

The results of the colchicine treatments showed that it was easier to obtain tetraploids by treating $F_1$ diploid seed than seed of homozygous varieties. Only 4 per cent of the treated seed of varieties produced tetraploid progeny compared to 18 per cent from the $F_1$ hybrid seeds.

TABLE 5.—MEAN PER CENT FERTILITY OF UNSELECTED $C_2$, $C_3$ AND $C_4$ GENERATIONS OF COLCHICINE-INDUCED TETRAPLOID BARLEY VARIETIES GROWN IN 1957

| Variety | $C_2$ | No. of plants tested | $C_3$ | No. of plants tested | $C_4$ | No. of plants tested |
|---|---|---|---|---|---|---|
| *Two-rowed* | | | | | | |
| Jet | — | | 71±3 | 2 | 55±11 | 2 |
| Sanalta | — | | 79±4 | 2 | 62±12 | 2 |
| *Six-rowed* | | | | | | |
| Gateway | — | | 34±3 | 16 | 39±6 | 8 |
| Montcalm | — | | 32±2 | 6 | 29±5 | 6 |
| Wolfe | 64±5 | 10 | 61±3 | 24 | 60±2 | 76 |
| OAC 21 | 62±5 | 6 | 62±4 | 10 | 59±5 | 10 |
| Vantage | 65±4 | 4 | 56±4 | 7 | 49±4 | 4 |

The fertility data of unselected tetraploid plants within the varieties and hybrids studied in six successive generations are presented in Table 1. The mean per cent fertility fluctuated within each variety and hybrid, with no apparent trend towards increased or decreased fertility with advancing generations of the hybrid material, but with some increase in the varieties. Tetraploid varieties were generally poor in vigour and the tetraploid variety Awnless did not survive. No tetraploids were produced from the variety Wong.

The differences in the distribution of fertility classes shown in Table 2 were highly significant statistically, which indicates that there was an association between the fertility of the $C_3$ parents and the $C_4$ progeny of the tetraploids derived from hybrids. The results also show that selection was more effective in tetraploids derived from hybrids than in those from the variety Herta. It was not possible to select any "above 75 per cent" or "50 per cent" fertile plants in the $C_3$ generation of the variety 40 Day.

The results of selection for fertility in the $C_3$ generation are presented in Tables 3 and 4, confirming the expectations regarding the efficacy of selections to improve the seed fertility of tetraploid barley.

The mean fertility of the $C_4$ progeny of the $C_3$ "above 75 per cent" selections was significantly higher than the mean of the "below 25 per cent" selections and also of the unselected $C_4$ progeny presented in Table 1. Similarly, the mean fertility of the progeny of the "50 per cent" $C_3$ selections was significantly higher than the mean of the "below 25 per cent" $C_3$ selections.

There was a significant difference between each of the $C_5$ means shown in Table 4. The fertility of the unselected $C_5$ generation of the hybrids shown in Table 4 grown in 1958 was 51 per cent. The general lowered fertility of tetraploids grown in 1958 was probably due to the dry conditions which prevailed during most of the growing season.

TABLE 6. — PER CENT FERTILITY OF $C_4$ TETRAPLOID AND DIPLOID BARLEY VARIETIES GROWN IN THE GREENHOUSE 1956-57 (MEAN OF 6 REPLICATES)

| Material | Tetraploid | Diploid |
|---|---|---|
| *Two-rowed* | | |
| Herta | 47 ± 4 | 93 ± 1 |
| Sanalta | 75 ± 5 | 91 ± 4 |
| Jet | 66 ± 2 | 94 ± 1 |
| Saitama[1] | 60 ± 7 | 93 ± 0 |
| Means of two-rowed | 62 | 93 |
| *Six-rowed* | | |
| Vantage | 29 ± 3 | 93 ± 1 |
| Gateway | 31 ± 6 | 87 ± 2 |
| Montcalm | 9 ± 1 | 75 ± 3 |
| Wolfe | 40 ± 7 | 80 ± 6 |
| 40 Day | 38 ± 2 | 96 ± 1 |
| OAC 21 | 43 ± 7 | 94 ± 1 |
| Means of six-rowed | 32 | 86 |

[1] $C_{12}$ introduced from Japan

TABLE 7. — MEAN PER CENT FERTILITY OF $F_2$ PLANTS FROM HYBRID TETRAPLOIDS GROWN IN THE GREENHOUSE DURING THE SPRING OF 1958 TOGETHER WITH THE $C_6$ GENERATION OF SANALTA

| Hybrid or variety | No. of plants tested | Fertility mean |
|---|---|---|
| Montcalm x (40 Day x Herta) | 2 | 15 ± 7 |
| (40 Day x Herta) x Saitama | 7 | 50 ± 6 |
| (40 Day x Herta) x (40 Day x Herta) | 4 | 31 ± 8 |
| Montcalm x Jet | 8 | 66 ± 3 |
| Saitama x Jet | 8 | 81 ± 3 |
| (Wong x Herta) x Sanalta | 5 | 57 ± 5 |
| Sanalta x (Wong x Herta) | 2 | 57 ± 7 |
| Saitama x (Wong x Herta) | 10 | 70 ± 1 |
| Montcalm x Saitama | 5 | 53 ± 8 |
| Sanalta x Saitama | 7 | 66 ± 4 |
| Herta x Sanalta | 8 | 59 ± 4 |
| Sanalta | 4 | 62 ± 7 |
| Mean | | 56 |

The fertility data of seven tetraploid varieties in which no selection for fertility were made are presented in Table 5. Seed for the $C_2$ generation was available for only three of the varieties. There was no apparent increase in fertility with advancing generation.

The results from the greenhouse fertility studies on the $C_4$ generation are presented in Table 6. In this study every spike was classified for fertility. The mean fertility of the diploid two-rowed varieties was also significantly higher than the six-rowed varieties.

Only 8 per cent of the 1200 florets of tetraploid crossed in the greenhouse set seed. These $F_1$ seeds were sown in the greenhouse and 48 per cent produced mature plants. The $F_2$ were also sown in the greenhouse and the fertility classifications on the plants produced are presented in Table 7. There was no apparent relationship between the fertility level of the parents and $F_2$ progeny obtained in this experiment.

TABLE 8. — MEAN PER CENT FERTILITIES OF $F_3$ PROGENIES FROM HYBRIDS BETWEEN TETRAPLOIDS GROWN IN THE FIELD IN 1958

| Hybrid or variety | $F_2$ "above 50%" | | $F_2$ "below 25%" | |
|---|---|---|---|---|
| | $F_3$ mean | No. of plants tested | $F_3$ mean | No. of plants tested |
| Montcalm x Jet | 54 ± 10 | 2 | 40 ± 3 | 59 |
| Saitama x Jet | 68 ± 5 | 18 | 55 ± 1 | 78 |
| (Wong x Herta) x Sanalta | 70 ± 2 | 52 | — | — |
| Saitama x (Wong x Herta) | 70 ± 2 | 20 | 51 ± 6 | 13 |

The fertility classification of two fertility selections made from the $F_2$ progenies of the earliest hybrids between tetraploids grown under field conditions is presented in Table 8. The "below 25 per cent" selections of (Wong x Herta) x Sanalta did not survive.

## DISCUSSION AND CONCLUSIONS

Selection for fertility appeared to be more successful in tetraploids derived from hybrids than in those from varieties. The fertility studies of hybrids between tetraploids indicate that selection for fertility in this material may also be effective.

Tetraploids produced from six-rowed varieties were lower in fertility than the tetraploids from two-rowed varieties. It was not possible to select for higher fertility in the six-rowed tetraploid variety, 40 Day. This agrees with the findings on six-rowed varieties reported by Reinbergs and Shebeski (8) and Ono (7). In the two-rowed variety, Herta, differences between the progenies of high and low fertility selections were not significant. Ono (7) reported an increase in fertility after seven generations of selection in several two-rowed varieties.

A study of Ono's results indicate that part of the apparent increase in fertility he reported may be due to different environmental conditions in later generations. Reinbergs and Shebeski (8) have shown that the environment does affect the fertility of tetraploids.

The fact that two-rowed varieties were higher in fertility than six-rowed varieties may be due to their competitive advantage for available nutrients, since the lateral florets are sterile. It might also be that two-rowed types are better adapted to chromosomal changes. It has been observed at the University of Alberta, by Kasha*, that two-rowed, heterozygous translocation lines were more fertile than similar six-rowed lines. Since nearly all wild species of barley are two-rowed this indicates that such types may be more tolerable to natural selection pressures.

The diploid varieties used in this study were considered to be homozygous. Therefore, the tetraploids obtained by doubling them should

*Unpublished.

also be homozygous. The possibility of selecting for higher fertility in such populations could only be based on the assumption of an unstable chromosome complement in early generations after doubling, which could be stabilized in later generations. Tetraploids derived from hybrid material would be heterozygous. Therefore, the fact that selection did not appear to be as effective in tetraploids derived from homozygous varieties as it was in tetraploids from hybrids indicated that fertility in tetraploid barley is genetically controlled. Müntzing (3) and Rommel (9) stated that the fertility in tetraploid barley obtained from hybrid material was higher than in parental tetraploids. The highest fertility reported by Rommel (9) for an individual plant was 69 per cent. In this study many plants had fertility values over 75 per cent. Part of the difference in fertility between the tetraploids derived from hybrids and those derived from varieties may be due to hybrid vigour as reported by Müntzing (4).

From reports in the literature and the data obtained in this study, it is concluded that hybridization and subsequent selection can be used to increase the fertility of tetraploid barley. Müntzing (3), working with two-rowed varieties, postulated that it should be possible to obtain highly fertile lines by using this method. It is likely that tetraploids derived from combinations between widely divergent stocks of diploids may be more valuable as a source of fertile selections than tetraploids from hybrids of closely related varieties.

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## NOTE ON A MULTI-CHANNEL INTEGRATOR FOR A RECORDING POTENTIOMETER

Physical variables encountered in agricultural research are being measured more and more by means of recording potentiometers. With such instruments large quantities of data can be recorded in a very short time. The time required to read the charts and to process the data may be many times greater. For many purposes, time-integrated values such as hourly solar radiation or hourly mean temperatures in digital form are the most desirable. Digital data systems* are commercially available but their large initial cost has prevented their application to problems in agricultural research. This note describes a low-cost attachment to a recording potentiometer which eliminates chart reading and provides time-integrated values in digital form for several channels.

To obtain integrated values the input signal to the recorder is alternately switched between a constant know signal (e.g., a zero signal) and the variable d.c. millivolt signal to be measured. When the input is switched from a zero signal to some non-zero signal the balance motor drives the pen carriage to a new position on the scale. The rotation of the balance motor, which is proportional to the signal, is recorded by a revolution counter. The revolution counter is then disengaged, the zero signal applied to the recorder input, and the process repeated. The revolution counter is always engaged before the input to the recorder is switched from the zero signal to the variable signal and disengaged before the input is switched from the variable signal back to the zero signal. After a sufficient time-interval and a sufficient number of pen-sweeps back and forth, the totalized count on the revolution counter is proportional to the time-integrated value of the measured variable.

A complete pen-sweep up to the balance point and back to zero can be considered as one sample of the unknown variable. One of the most important aspects of accurate time-integration of a variable signal by discontinuous sampling is the sampling frequency. The required frequency of sampling depends on the true variability of the physical variable, the time constant of the transducer converting the physical variable to an electrical signal, and the balance-time of the recorder. The signal is completely specified if Shannon's Sampling Theorem (1) is obeyed. That is, if the highest frequency component of the signal from the transducer is $f$, then the frequency of sampling must be $2f$ to completely specify the signal. For example, if a fast-response thermocouple is the transducer, air temperature should be sampled much more frequently than soil temperature. For a more complete discussion on this aspect of sampling the reader is referred to a paper by Tanner (2).

For a signal which may vary between positive and negative values (e.g., net radiation), integration may be obtained by placing the electrical zero of the recorder on-scale. Then the recorder will move in either direction away from zero, depending on signal polarity, to the new balance

*e.g., Datex Corp., Monrovia, California.

point. However, for near-zero signals the integrated value may contain relatively large unknown errors because of the gear-lash in the revolution counter coupling and the recorder dead-band error. Such errors can be eliminated by supplying, instead of the constant zero signal so far described, a constant negative signal which will drive the recorder to the end of the scale with the zero still on-scale. The recorder then approaches the balance point for both positive and negative signals always from one direction. The dead-band and gear-lash errors can then be eliminated.

In order to obtain multi-channel integration it is necessary to engage a separate counter for each different signal. For this purpose a six-pole stepping switch is used to select the signals going into the recorder and to select a solenoid to engage the correct counter. Mismatching of a signal and its appropriate counter is impossible because the selecting contacts are controlled by the same shaft. Gold-plated double-pole contacts are used for each of the millivolt leads. Shielding of the millivolt leads and contacts is essential.

The arrangement of the recorder and attachment for multi-channel integration is shown in Figure 1. An appropriately energized solenoid (*d*) pulls a pivoted counter (*c*) against the counter-drive shaft ($s_1$) so that the knurled brass wheel (*b*) of the counter engages a rubber wheel (*w*) on the counter drive shaft. This type of drive was found superior to a toothed-gear drive. A spring (*g*) ensures that the counter wheel is disengaged from the drive wheel when the solenoid is not energized.

The counter drive shaft is coupled by means of a short length of heavy-walled rubber tubing (*t*) to a second shaft ($s_2$) protruding from a bearing in the rear of the recorder housing (Figure 1). The rubber tubing serves as a universal joint and allows shaft $s_2$ to be slid back and forth in its bearing so that the recorder chassis may be swung open. Figure 2 shows the interior of the recorder with the chassis swung open. When the chassis is closed, the two prongs (*p*) on the end of the shaft $s_2$ are engaged into the two small holes of the brass receptacle (*r*) mounted on a gear driven by the balance motor pinion (*m*).

The counter assembly, along with the time and date, are photographed periodically by a time-lapse movie camera. By the use of electrical read-out counters* automatic printing or punching of integrated digital data is possible.

The arrangement of timer cams and the electrical circuits used to control the sequence of operations is shown in Figure 3. It should be noted that the three cams are on a common shaft of a synchronous motor in the relative rotational positions shown. When a microswitch actuator drops into a notch on a cam, then the switch is in the normal position. A micro-switch may be wired so that a circuit is either normally open (NO) or normally closed (NC).

When the microswitch on the No. 1 cam closes, the rotary solenoid of the selector switch is energized, the selector switch advances one position,

*e.g., Veeder-Root 1504.

FIGURE 1. View of revolution counter and solenoid assembly attached to recording potentiometer. Symbols are explained in text.

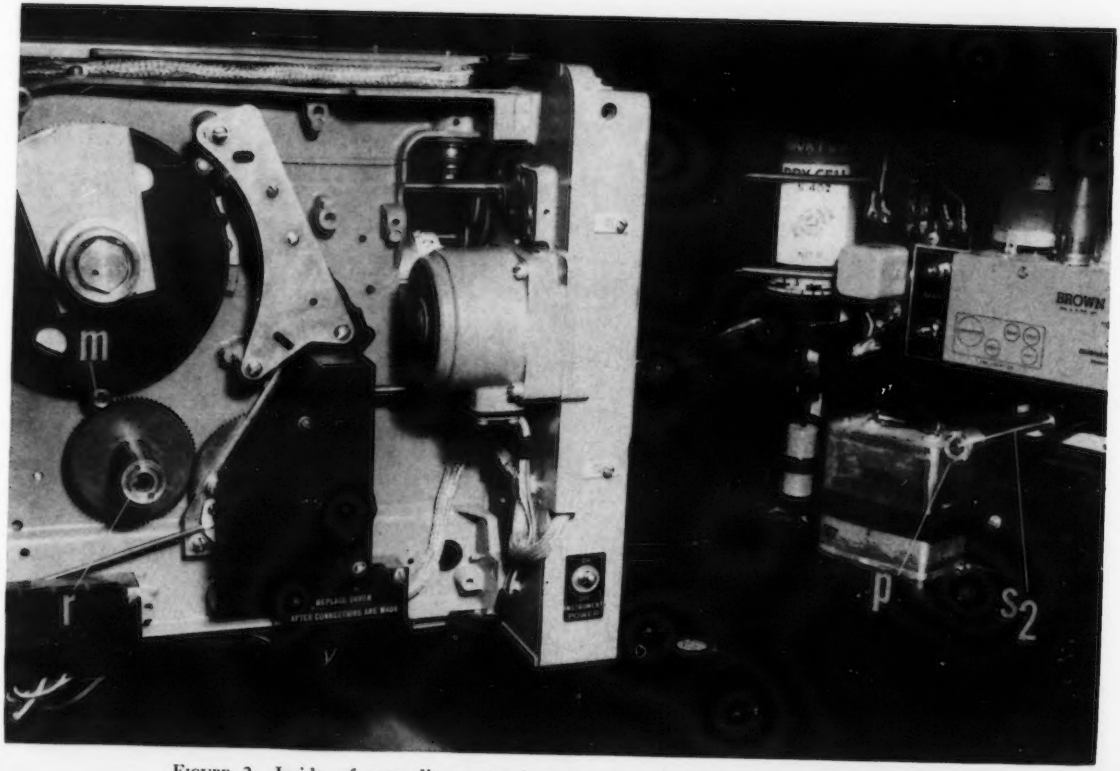


FIGURE 2. Inside of recording potentiometer case. Symbols are explained in text.

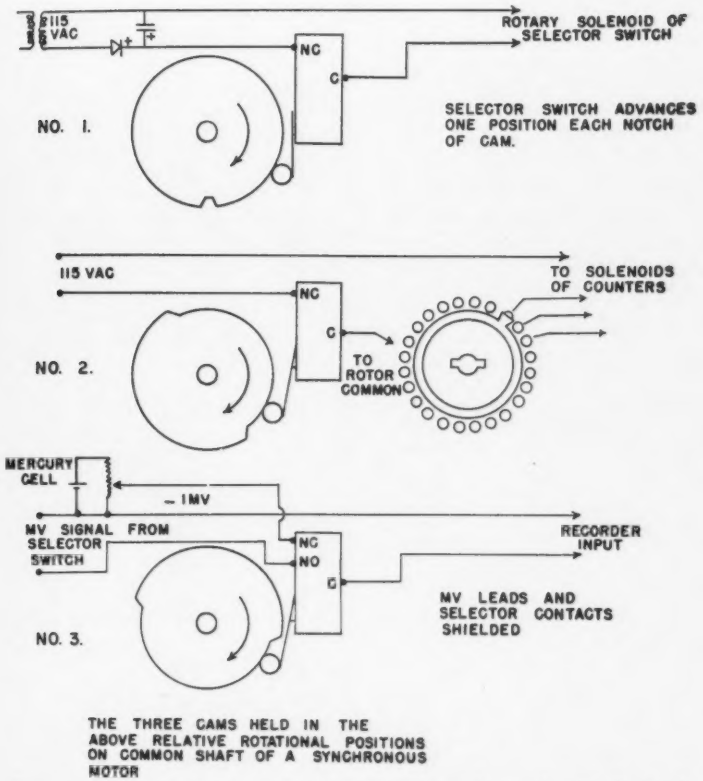


FIGURE 3. Control circuits used in the integration of several millivolt signals.

and a new variable is put across the recorder input. Then in sequence:

(1) No. 2 switch closes and a counter wheel is engaged. The particular counter wheel depends on the position of the selector switch.

(2) No. 3 switch goes to the non-normal position, the unknown signal is applied and the potentiometer reaches the new balance point.

(3) No. 2 switch opens and the counter wheel is disengaged.

(4) No. 3 switch goes to the normal position, the end-of-scale negative signal is applied and the potentiometer drives to the end of scale. Then the No. 1 switch closes, a new variable is selected and the sequence is repeated. In Figure 3 the cams are between (1) and (2) in the sequence.

The desired total or average value of any variable in the proper units for any period of time is obtained by multiplying the difference between the counter reading at the beginning and the end of the period by a factor which depends on the calibration of the measuring instrument, the number of samples of that variable in the period, the recorder span, and the number of counts obtained per full-scale pen sweep. Also, a constant value must be subtracted depending upon the value of the negative zeroing signal used. To be able to eliminate the error caused by a significant zero drift

of the recorder, a zero signal (short-circuit) should be periodically measured and integrated. In this device 25 counts for each full-scale pen-sweep were obtained.

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